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DONALD RUSSELL HOOKER

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No. 1

## THE ALKALI TOLERANCE OF THE DOG HEART<sup>1</sup>

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The isolated dog heart has been shown to function without significant impairment when perfused with physiological solutions of varied pH values between 7.0 and 7.8 (1). Recently it was demonstrated that the dog heart in situ may function without serious impairment until a blood pH value of 6.25 is attained by infusion of hydrochloric or lactic acid into the circulating blood (2). This value approaches that found for the isolated, perfused frog heart (3). Not only is the acid tolerance of the isolated frog heart greater than that of the isolated dog heart, but accepted limits of alkali tolerance in isolated hearts are much higher in the frog than in the dog heart. Mines (3) found that the frog heart ceased to beat at  $\text{cH } 10^{-10}$ . No significant changes were noticed until  $\text{cH } 10^{-9}$  was reached, when the heart accelerated only to slow again as  $\text{cH}$  was increased, with abbreviation of the QRS and P-R intervals. Clark (4) reported that at pH 8.5 the perfused frog heart showed signs of arrhythmias and failure in conduction, followed by a cessation of beat if the perfusion were continued. Both Mines and Clark, in confirmation of the earlier work of Gaskell (5), found systolic arrest as the terminal event in perfusion with alkaline solutions. The only work on the alkali tolerance of the isolated mammalian (dog) heart is that of Andrus and Carter (1) who showed that under optimum perfusion conditions the heart failed at a limiting pH of 7.8. Prior to final arrest, alkalinity increased the rates of impulse formation and conduction as shown by the increased rate and abbreviated P-R and QRS intervals. The present investigation was undertaken to determine the alkali tolerance of the dog heart in situ.

In the course of the present series of experiments, in some of which the pH of the circulating blood was raised to 8.4 before the heart began to fail, it was noticed that the animals which tolerated the greatest quantities of added alkali per kilogram of body weight were those in which there was obvious muscular activity. This began with fibrillary muscle twitching passing into rigidity, especially of the chest muscles, and was followed in extreme cases by a rigidity

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extending throughout the whole skeletal musculature. This suggested that in these extreme conditions a greatly increased lactic acid production by the muscles was reinforcing the primary buffers of the blood in combatting pH change due to added alkali. Accordingly in later experiments, determinations of blood lactate were carried out concurrently with the electrocardiographic and pH determinations.

**MATERIALS AND METHODS.** The methods were essentially those employed in the previous investigation (2).

Experiments were performed on 24 dogs varying in weight between 3 and 22.5 kgm. The anesthetic was nembutal, 30 mgm. per kgm. administered intravenously. Preparation of the animals consisted in inserting a tracheal cannula, exposing the femoral blood vessels in both hind legs and tying an infusion cannula into one femoral vein.

With alkali infusion, in contrast to acid infusion, the natural respiratory movements became depressed during the experiment. To avoid as far as possible the complicating effects of anoxia on the heart, artificial respiration was started at the first sign of respiratory depression.

Aqueous solutions of NaOH were infused into the femoral vein, using different concentrations in the several experiments. The most satisfactory concentration proved to be 0.3N and it was therefore used in 15 experiments. In one experiment in which 0.15N NaOH was used, the final stages of the experiment were complicated by pulmonary edema. A concentration of 0.5N used in eight experiments was satisfactory if administered sufficiently slowly, but five out of the eight animals infused with it died during the initial stages of the experiments. This was apparently due to too rapid an initial perfusion rate since two successful experiments were carried out using this strength. A similar lethal effect of strong acid was found in the earlier work (2). In the alkali experiments, as in the acid, these early heart failures usually occurred with but slight changes in pH. Experience showed that it was not safe to administer the first 50 cc. of alkali at a rate greater than 0.06 mM/kgm./min. if the animals were to be carried over the first stages of the experiment. After this initial stage the rates of injection were varied to give overall injection rates varying from 0.06 mM/kgm./min. to 0.5 mM/kgm./min. Some of the dogs, however, tolerated a higher initial infusion rate.

Blood samples for pH determination were drawn without stasis from the femoral artery of the leg not used for infusion. Samples for lactate determinations were drawn from either femoral artery as convenient. The methods were those already described (2), using a Beckman glass electrode apparatus for pH determinations and the Koenemann technique for blood lactate (6).

Electrocardiograms were taken from lead II at intervals corresponding to successive increments of 50 cc. of infusion fluid, using a Sanborn cardiette with standard sensitivity. Blood samples for pH and lactate determinations were taken simultaneously with the electrocardiographic records. In the terminal stage of the experiments all three determinations were often made at shorter intervals, in accordance with unusual changes in the electrocardiogram observed

in the cardioscope. Throughout this paper the pH and lactate values given as final are those immediately preceding disintegration of the cardiac complex, i.e. while the electrocardiogram showed neither complete heart block, nor prolongation of intraventricular or auriculo-ventricular conduction time.

**RESULTS.** 1. *Changes in pH and lactate; total concentration of alkali tolerated; muscular responses.* The experiments fall into three groups; a group in which the dogs died in the initial stages of the experiment, and two well-marked groups of longer survival, distinct from each other as to the pattern of pH changes of the blood, muscular responses, increases in blood lactate, and the total quantity of alkali tolerated before the heart finally failed. The electrocardiographic results do not fall similarly into groups and will be dealt with in a separate section.

*Group I.* In this group of nine animals the heart failed in the initial stages of the experiment either because of a high initial rate of alkali infusion in terms of mM/kgm. of body weight, or because of some individual sensitivity to alkali. There was in all these cases severe anoxia, the blood becoming exceedingly dark in colour. These heart failures were not correlated with the concentration of alkali in the animal, since greater quantities were administered in the succeeding two groups of experiments. In no case was more than 100 cc. of alkali infused, failure occurring at mM/kgm. body weight values of NaOH of 5 mM. or less and in most cases at comparatively low pH values, in five of the nine experiments below pH 7.70.

*Group II.* In this group the overall infusion rates were low, varying in different experiments between 0.06 and 0.1 mM/kgm. of body weight per minute. None of this group showed any obvious sign of muscle rigor. The blood lactate did not rise above 100 mgm. per cent. The hearts of these animals failed at lower total mM/kgm. values of added alkali (8.0–15.0 mM/kgm. body weight) than did those in group III and the blood pH immediately previous to heart failure (7.7–7.93) was lower than in the latter group.

*Group III.* The overall injection rates in this group were higher than in group II, varying in different experiments from 0.13–0.5 mM/kgm./min. All the animals in this group showed rigor of the respiratory muscles which made artificial respiration necessary, although some natural respiratory movements persisted except where chest rigor was extreme. Subsequently, in some cases, other muscle groups were involved as well. In one experiment the rigor extended throughout the whole skeletal musculature. Associated with rigor, there was a great increase in blood lactate which rose to values of from 101 to over 200 mgm. per cent. The added alkali tolerated before final failure of the heart varied from 12.2–52 mM/kgm. body weight and the blood pH always rose to higher values (8.12–8.40) than those previously reported as tolerated by the dog heart.

There was great variation in individual responses in both these groups. The first infusions of alkali resulted in a rise in pH culminating in a temporary maximum (referred to in table 1 as the *first maximum*). After this maximum, the patterns of pH changes in the two groups diverged. In group II, after a sharp fall from the first peak, the pH rose immediately, the rise culminating in failure of the heart. In group III, after a similar fall from the first maximum, the pH

was maintained at a varying, but lower level during the addition of successive amounts of alkali, till a final rise in pH occurred with failure of the heart.

TABLE 1

DOG NO.	WEIGHT	INFUSION RATE	INITIAL		AT FIRST MAXIMUM			FINAL		
			pH	Lactate	pH	NaOH	Lactate	pH	NaOH	Lactate
	kgm.	mM/kgm. min.		mgm.%		mM/kgm.	mgm.%		mM/kgm.	mgm.%
1	8.5	0.19	7.40		8.20	10.6		8.13	17.8	
2	10.0	0.24	7.54		8.15	15.0		7.70	29.4	

## Group 2

17	15.0	0.08	7.46	16.0	7.86	4.0	22.0	7.91	8.0	69.0
18	21.0	0.06	7.42	6.6	7.88	5.0	47.0	7.93	10.0	53.0
20	22.5	0.10	7.58	13.0	7.88	6.6	70.0	7.71	9.2	90.0
24	7.3	0.08	7.56	9.0	7.77	5.2	between* 35.0-65.0	7.90	15.0	between 88.0-108.0

## Group 3

5	8.0	0.30	7.55		8.00	13.2		8.35	30.8	
6	8.0	0.40	7.47		8.06	18.7		8.30	52.0	
8	3.0	0.50	7.55		8.20	12.5		8.35	45.0	
13	13.5	0.13	7.60	40.0	8.12	4.5	84.0	7.93	12.2	between 188.0-252.0
14	10.5	0.19	7.51	16.0	7.94	18.5	77.0	8.40	38.5	225.0
21	16.0	0.15	7.62	23.0	7.89	4.6	50.0	8.40	20.6	between 174.0-215.0
23	6.8	0.23	7.49	12.0	8.33	9.9	25.0	8.12	16.5	101.0

\* In a few instances lactate determinations were not made at the pH reported for certain events in the experiments. In these cases the determinations immediately preceding and succeeding the event are given. Since lactate progressively increased during the course of the experiments, the true value will lie between these.

In spite of the individual variations, groups II and III form two contrasting series of experiments. The differences are tabulated below. These differences in response are correlated with the rate of alkali injection.

	Group II	Group III
Injection rates ...	0.06-0.10 mM/kgm./min.	0.13-0.5 mM/kgm./min.
Rigor.....	No obvious rigor	Rigor
pH at first maximum.....	7.77-7.88	7.89-8.33
pH before final failure of heart.....	7.71-7.93	8.12-8.40 (with the exception of 1 experiment where a lower pH of 7.93 was recorded)
Alkali added before heart failure.....	8.0-15.0 mM/kgm.	12.2-52 mM/kgm.
Blood lactate before heart failure.	53-100 mgm. per cent	101-225 mgm. per cent

In two of the group III dogs, heart failure occurred at a pH lower than that at the first maximum, in the other five dogs of this group, at a higher pH than the first maximum. The onset of rigor occurred either before or after the first maximum was reached, but not in these experiments at a pH lower than 7.81. Once initiated, it persisted and increased during the period of lower pH values following the first maximum and disappeared only at the time when the heart began to fail.

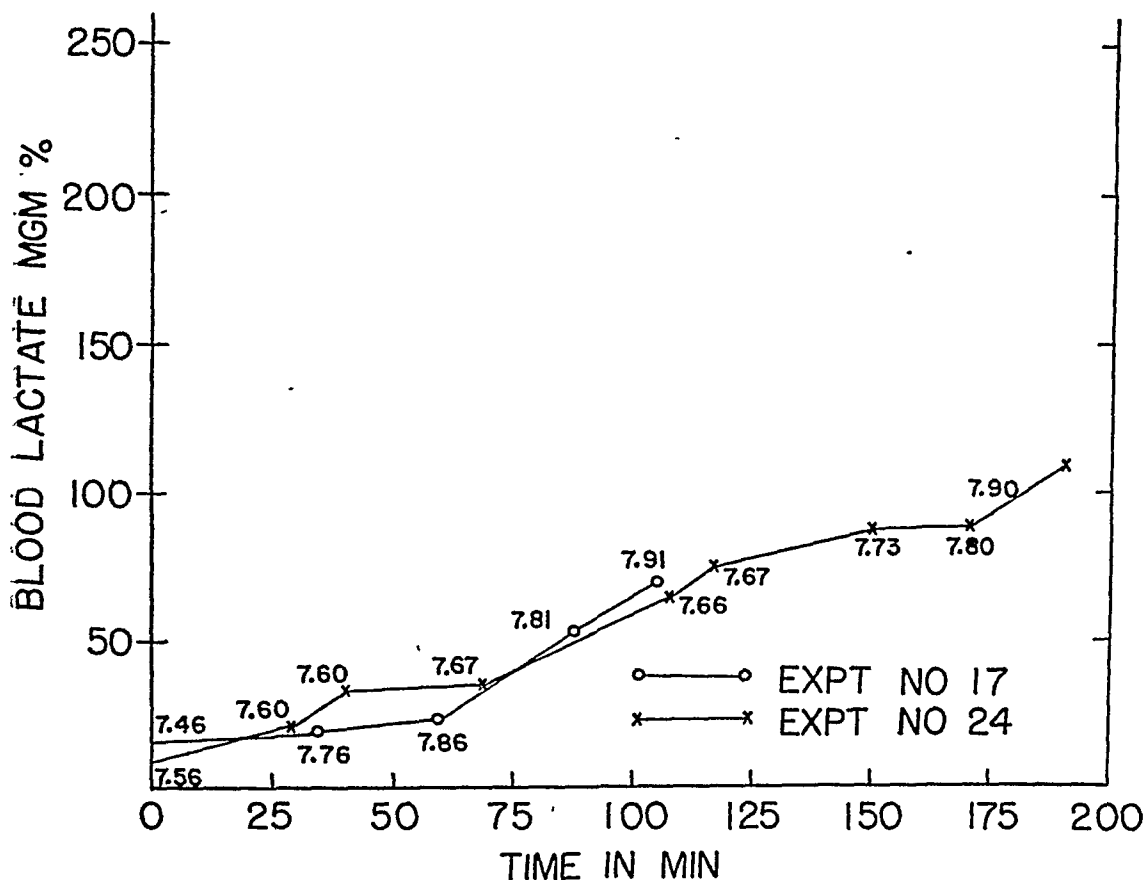


Fig. 1. Blood lactate changes in two dogs of group II. The blood pH values determined at the same time as the lactate concentrations are marked on the curves. The heart of dog 17 failed at pH 7.91, that of dog 24 at pH 7.90.

*Miscellaneous group.* Experiments 1 and 2 are not included in the above groups since at this early stage the development of rigor was not being watched. Experiment 7 was discarded because of errors in pH determinations. Experiment 4, in which HCl was infused during the experiment, is also omitted from the grouping.

*Lactate changes.* Blood lactate changes were followed in four dogs in group II and four in group III. Two experiments in each group are plotted in figures 1 and 2 which show the comparatively small and slow changes in blood lactate in the group II dogs in contrast to the large, and in the later stages of the experiments, rapid increases in group III dogs. Individual variations in the animals were such that no definite correlation was found between lactate and either pH or mM/kgm. of base added. The significant features of the lactate results are:

1. High lactate concentrations occurred only in those dogs developing obvious muscle rigor. 2. High lactate concentrations were associated with survival of the hearts to the most alkaline pH values.

*Blood pressure.* The blood pressure was well maintained until the final failure of the heart in the three experiments in which it was recorded. The records were similar for dog 8, which showed well-marked rigor and for dogs 17 and 18 in which there was no obvious rigor. The blood pressure remained at approximately the original level until the time of the first maximum in the pH curve, at

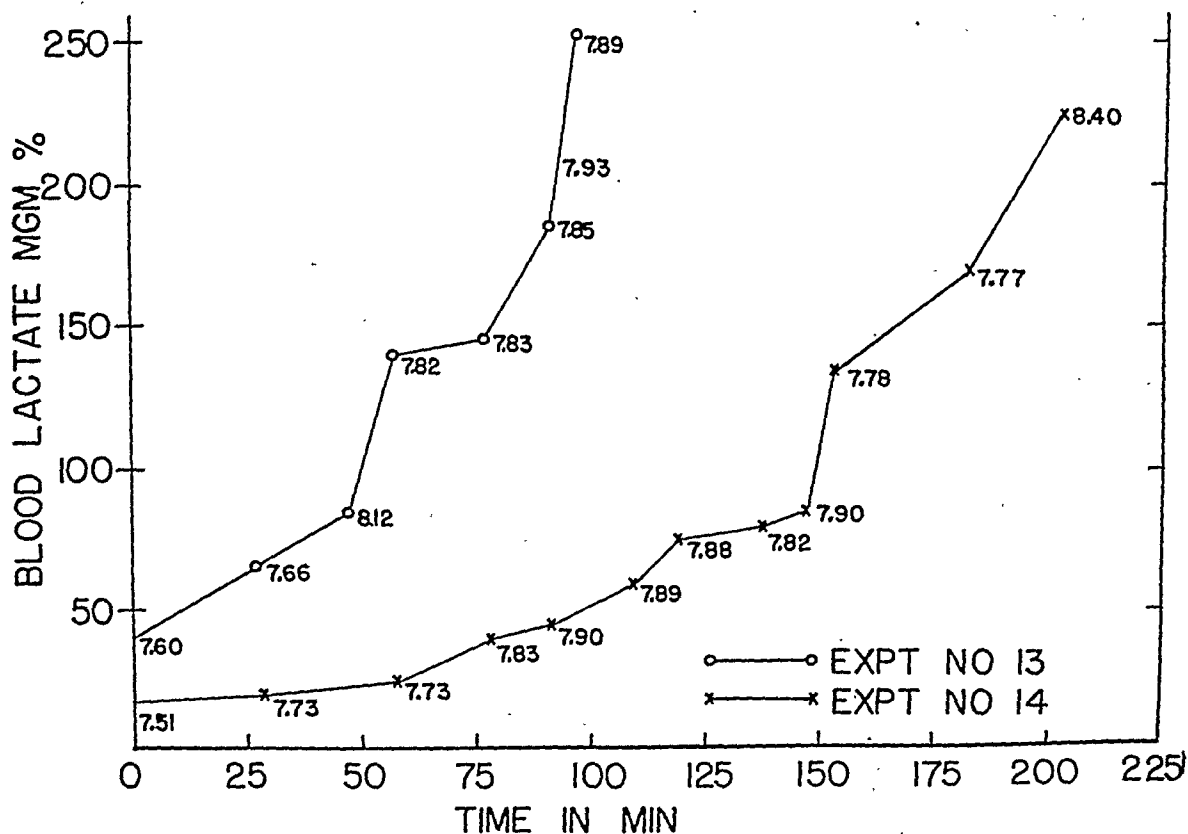


Fig. 2. Blood lactate changes in two of the group III dogs. Blood pH values obtained at the same time as the lactate determinations are marked on the curves. Failure of the heart occurred at pH 7.93 in dog 13, at pH 8.4 in dog 14. Rigor onset was observed at pH 7.82 in dog 13, at pH 7.83 in dog 14.

which time artificial respiration was started in these three experiments. At this stage there was an immediate fall to a plateau at 60 to 90 mm. Hg with no further fall until the sharp decrease to zero concurrent with the failure of the heart.

*The electrocardiogram.* Electrocardiographic changes were not as regular with increases as they were with decreases in blood pH. The following are the most significant electrocardiographic changes encountered during the infusion of alkali.

1. *The T wave.* Depression of the T wave was the most constant electrocardiographic change encountered in this study. From the onset of most experiments, a diminution in the amplitude of the T wave occurred which progressed as the pH continued to rise, culminating in a complete reversal of polarity either before or

at the time of the first maximum pH (fig. 3). In one experiment the T wave was negative at the onset and increased progressively in depth as the pH rose. Increases in the T wave were occasionally noted either as a terminal event or prior to the institution of artificial respiration; these were probably due to cardiac anoxia rather than to variations in blood pH.

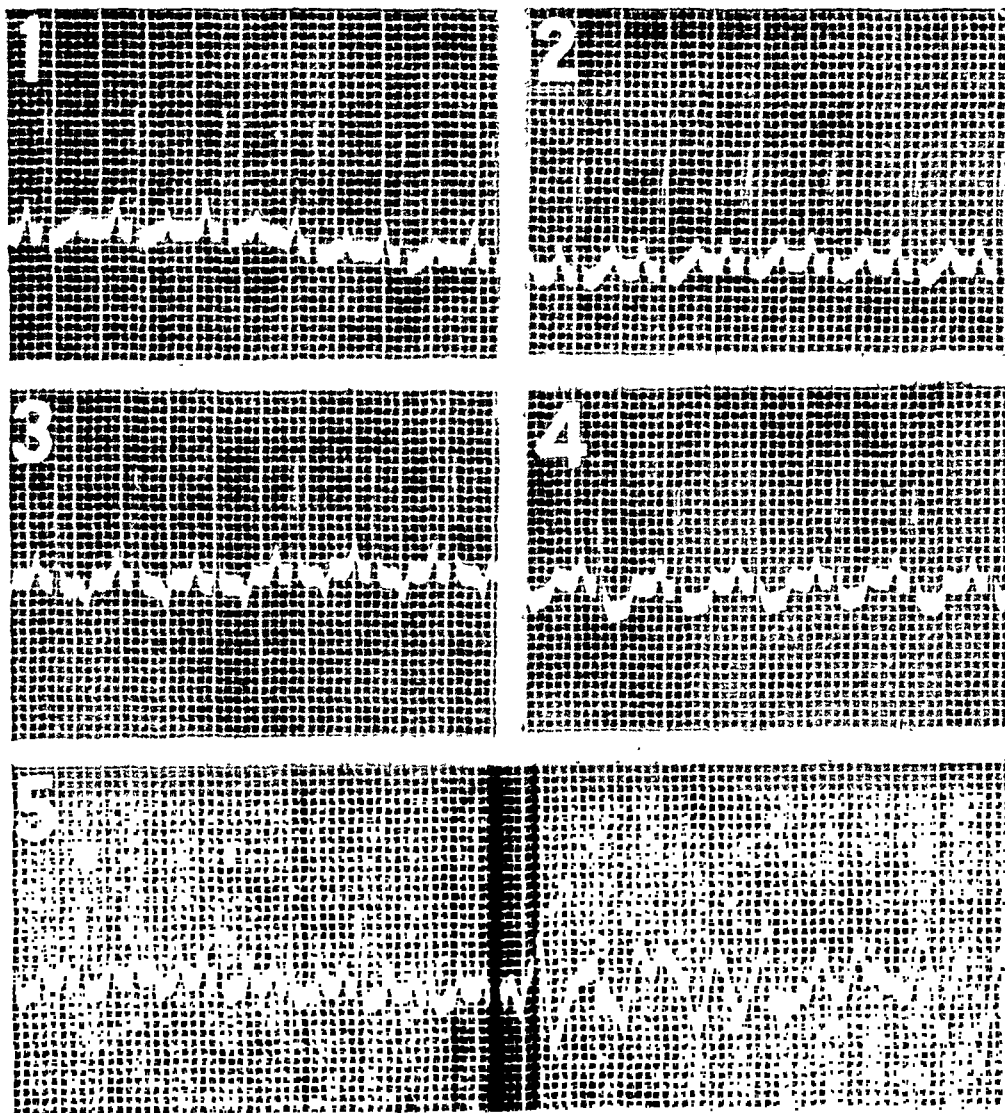


Fig. 3. Records from experiment 13. Record 1, at an initial pH of 7.60. Record 2, at pH 7.62 shows a slight increase in amplitude of the T-wave which is followed in record 3, at pH 8.11, by the characteristic reversal of polarity. Record 4, at pH 7.82, shows the RS-T depression which is succeeded in record 5, at 7.93, by ventricular fibrillation.

2. *RS-T deviation.* Seven instances of RS-T depression and one instance of RS-T elevation were observed. As in the acid experiments, the appearance of the RS-T depression usually indicated serious interference with cardiac function, and heart failure occurred shortly thereafter. No correlation was found in these experiments between the development of rigor and RS-T depression. The hearts of those animals which showed RS-T depression always passed into ventricular fibrillation suddenly as shown in figure 3. Ventricular fibrillation, however, occurred in some experiments without a preceding RS-T depression.

3. *The R wave.* In the majority of experiments, the R wave was unaffected throughout the early stages of infusion. Only after the T-wave depression had approached its maximum did the R-wave begin to decline in amplitude. The progressive decrease in the R wave from this stage on was an additional indication of impending cardiac failure (fig. 3).

4. *Rate.* The infusion of alkali produced no regular changes in rate. The effects varied not only in different experiments, but during the course of a single experiment. Furthermore, a comparison of initial rates and rates just prior to cardiac disintegration did not show any consistent variation; in the final stages of

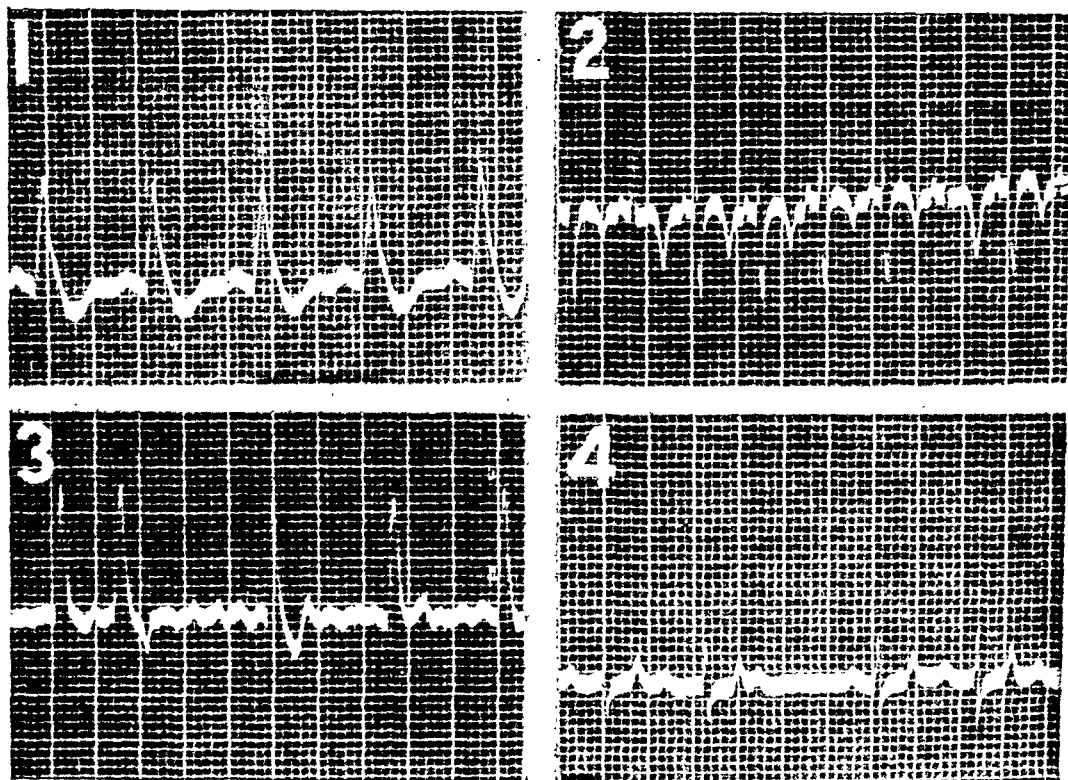


Fig. 4. Abnormal electrocardiographic patterns encountered during the infusion of alkali. Record 1, prolonged intraventricular and auriculo-ventricular conduction time. Record 2, pulsus polygeminus. Record 3, auricular fibrillation and ventricular extrasystoles. Record 4, partial auriculo-ventricular block. Records 1 and 4 are terminal records.

the experiments the rate was unchanged from the initial rate in three dogs, decreased below the initial rate in six and increased over the initial rate in seven.

5. *Conduction time.* Intraventricular conduction time was unchanged in all but two experiments; in these two it was abbreviated slightly. Auriculoventricular conduction time was increased in four experiments, decreased in six and remained unchanged in five. The abbreviation or prolongation was never more than 0.04 second and in the majority of cases less than 0.02 second. These variations scarcely exceed those found in normal dogs. Auriculoventricular block appeared only as a terminal event (fig. 4).

6. *Bazett's formula.* It has been shown by Bazett (7) and confirmed by Carter

and Andrus (8) that the value of  $K$  in the formula  $K = \sqrt{\frac{Q - T}{R - R}}$  is increased in tetany by the prolongation of the Q-T interval. There was no consistent increase in  $K$  in the present experiments even in the dogs with obvious rigor. In the sixteen experiments in which  $K$  could be calculated, the value was unchanged in three, decreased in five and increased in eight.

*Respiratory failure.* With the exception of the group I animals, increase in blood alkalinity to pH 7.8 had no noticeable effect on respiratory movements, but at or slightly above this value, a progressive decrease in both rate and depth of movement set in necessitating the use of artificial respiration. The depression of respiration was especially marked in experiments in which there was rigor of the chest muscles, but rarely progressed to complete suppression of natural respiratory movements. Irregular chest movements were observed in three experiments at blood pH values above 8.0.

*Mode of heart failure.* In six experiments ventricular fibrillation appeared suddenly in apparently well-functioning hearts showing only moderate RS-T depression. Ventricular fibrillation as the terminal event followed anoxia of several minutes' duration in one experiment.

In the remaining animals electrocardiographic complexes persisted at a progressively slower rate after it became clear that circulatory failure had occurred; it was assumed that the precipitating factor was failure of myocardial contractility. As a terminal electrocardiographic event, intraventricular block appeared in several of these experiments culminating in the development of monophasic complexes (fig. 5). In one experiment the chest and pericardium were opened and attempts made to obtain records using Wilson's (CV) lead. Monophasic complexes of large magnitude were recordable only when the heart electrode was placed on the apex of the left ventricle. In this instance the anterior surface became negative with each complex, the posterior surface showing an opposite sign.

In contrast to the widely dilated hearts found at autopsy after failure from acid intoxication, the six hearts which were examined after the alkali experiments appeared to be of normal size.

In the later stages of three experiments the whole diaphragm pulsed rhythmically in phase with the beat of the heart. The phrenic nerves passing over the pericardium were apparently stimulated by the spread of cardiac action currents. It is suggested that this abnormal stimulation of the phrenic nerves can be attributed to the lowering of the threshold of nerves in alkaline media (9). This phenomenon is another example of the classical demonstration of Kölliker and Müller (10) that cardiac action currents are of sufficient magnitude to stimulate somatic nerves.

**DISCUSSION.** The limits of alkali tolerance of the heart in these experiments are higher than those reported for the isolated perfused dog heart and approach more closely the limits found for the perfused frog heart. It was suggested in a previous paper (2) that the increased acid tolerance of the dog heart in situ, com-



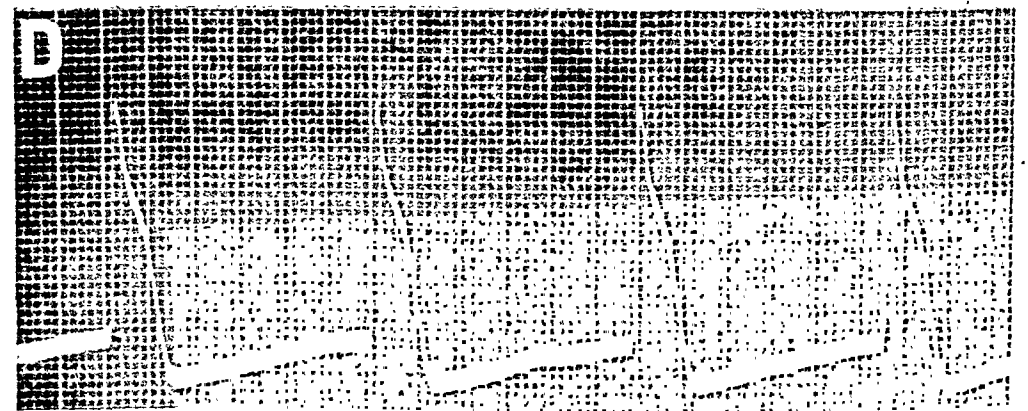
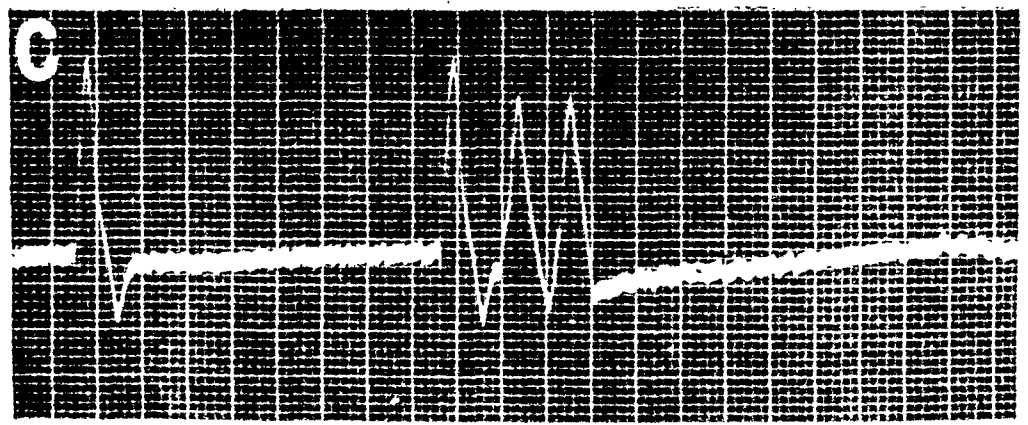
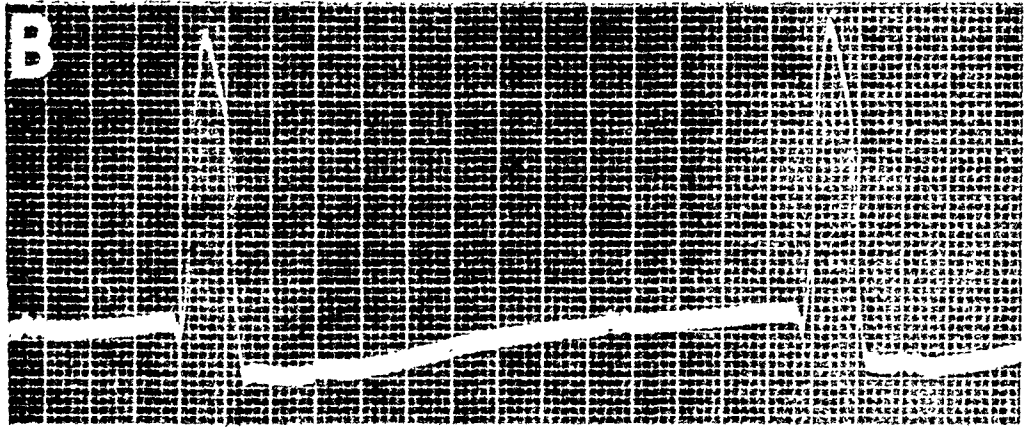
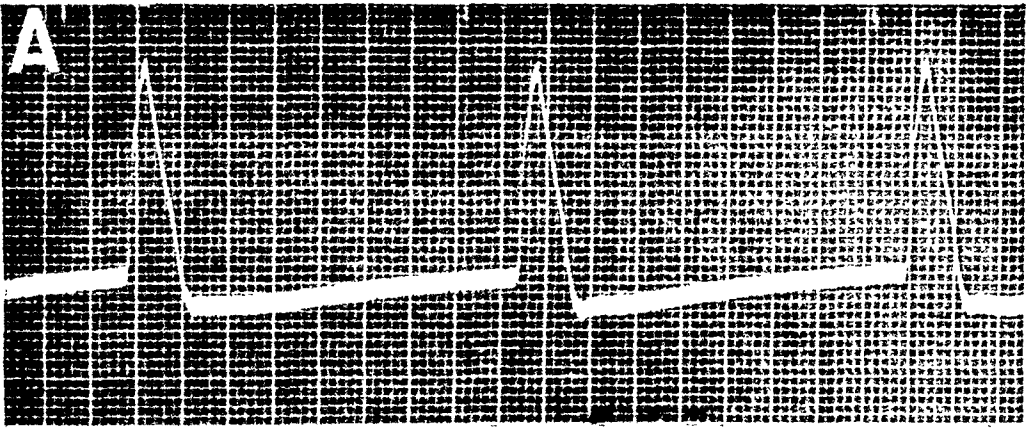


Fig. 5. Monophasic complexes in four experiments. Record D was taken with lead CV with the electrode on the anterior surface of the left ventricle. Records A-C were taken with lead 2.

pared with the isolated perfused heart, might be attributed to some degree of anoxia in the latter. In the present experiments, the introduction of alkali so depressed the respiratory movements that artificial respiration was necessary. Under these conditions it is improbable that there was any severe anoxia of the heart, since up to the moment of final failure the auriculo-ventricular dissociation characteristic of oxygen deficiency was absent. It is, therefore, reasonable to suggest better oxygenation of the blood in the intact animal as a factor influencing the greater tolerance to alkali. The question is, however, complicated by the finding that the hearts which survived the highest alkaline values were those in which the lactate concentration of the blood increased to high levels. In none of the hearts of animals in which the blood lactate remained below 100 mgm. per cent was the blood pH at any time higher than 7.97. In the group III animals with higher blood lactates, the highest pH values during the experiment were 8.12, 8.33, 8.4 and 8.4. In the other animals of this rigor group the maximum values were 8.3, 8.35 and 8.35.

No explanation suggests itself for this increased alkali tolerance of the hearts in dogs in which the overall infusion rate was rapid in terms of mM/kgm. of body weight; this is the high blood lactate group. Too high an initial rate resulted in early failure of the heart at comparatively low pH values. The use of the higher concentrations of acid similarly resulted in rapid heart failure in the earlier experiments, again without any great changes in blood pH. In the experiments with alkali there appears to be a time lag before the reactions set in which later result in the survival of the heart. During this initial period, slight increases in alkali may result in early failure of the heart. In no experiment was artificial respiration instituted at the start of the experiment so that it is impossible to rule out severe anoxia as the cause of these early deaths. In the later stages of the experiments, a rapid increase in the rate of alkali infusion was necessary to provide an adequate stimulus for the great increases in blood lactate which were associated with high alkaline tolerance of the hearts. In these later stages the time factor does not appear to be significant. Dogs of group II survived for 94-194 minutes of infusion, dogs of group III, 93-201 minutes, in spite of infusions of very different amounts of alkali and in spite of great differences in the pH values attained. It seems evident from these experiments that the limiting factor was not pH alone, but a combination of two or more factors, at least one of which was associated with the level of blood lactate.

Increases in blood alkalinity, in some cases as high as 8.43, did not depress the rhythmicity nor the excitability of the heart. The magnitude of the P-R and QRS changes was well within the limits accepted for the normal heart. It is, however, probable that intraventricular conduction is facilitated since in no experiment was a prolongation of the QRS interval recorded, and in two experiments out of sixteen analyzed it was abbreviated. There was no consistent effect of alkali on the heart rate, even during the course of single experiments.

The contractility of the ventricles was apparently unimpaired till the terminal stages of the experiment. After a drop in blood pressure coincident with the start of artificial respiration, the blood pressure was maintained at a lower but fairly constant level until the heart failed.

The predominant change in the electrocardiogram was a diminution and subsequent inversion of the T-wave. The same effect of alkali on the T-wave has been reported in experimental animals (11) and in the human (12, 13). This was frequently followed by depression of the RS-T segment. As in the experiments with acid infusion (2), the RS-T depression was of such magnitude that it could not be accounted for on the basis of alterations in the ventricular gradient. It must therefore be considered to be due to the development of an injury current in the right ventricle (14).

The R-wave was reduced in amplitude in the terminal stages of these experiments, in contrast to the acid experiments in which the amplitude of the R-wave increased. Harris (15) has suggested that an augmentation in ventilation will result in a decrease in amplitude of the R-wave. In these experiments it is impossible that the R-wave decreased as a result of increased ventilation, since some of the dogs had been under artificial respiration for over an hour before the diminution of the R-wave occurred.

It is possible that the single and multiple extrasystoles and auricular fibrillation recorded in the later stages of these experiments (fig. 4), as well as the terminal ventricular fibrillation, are due to spontaneous firing of cardiac pacemakers analogous to the spontaneous firing of nerve in alkaline media (9).

#### SUMMARY AND CONCLUSIONS

1. The most alkaline limit of blood pH tolerated by the hearts of fifteen normal dogs in situ was pH 8.4. This high tolerance of blood alkalinity was associated with an increase in blood lactate to over 100 mgm. per cent, usually to about 200 mgm. per cent. When the blood lactate level remained below 100 mgm. per cent the alkali tolerance was lower; the highest limiting pH found in these cases was 7.97.

2. The development of high blood lactate levels was associated with obvious activity of the skeletal muscles, especially those of the chest. In extreme cases the whole skeletal musculature passed into a state of rigor during the course of an experiment.

3. The most constant electrocardiographic change was a decrease in amplitude of the upright T wave which later culminated in a complete reversal of polarity.

4. Depression of the RS-T segment and decrease in amplitude of the R-wave occurred as unequivocal signs of impending cardiac failure; ventricular fibrillation always followed RS-T depression.

5. The progressive increase in blood alkalinity did not significantly affect the rate, the QRS or P-R intervals, nor did it produce heart block except as a terminal event.

6. Contractility was apparently not affected except in the terminal stages.

7. The respiratory mechanism was impaired at limits of blood alkalinity well below those for the heart. At about 7.8 this impairment was such as to necessitate the use of artificial respiration.

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# THE MEASUREMENT OF THE STROKE VOLUME FROM THE PRESSURE PULSE<sup>1</sup>

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It was proposed by Erlanger and Hooker (1) that the pulse pressure be regarded as a rough index to the stroke volume. Reasonably enough, the larger the ejection the greater the pressure rise. The relationship between the two cannot be close, however, for blood which leaves the heart during systole distends the arterial tree to different degrees and at different times in its several parts. The amount by which this distention raises the pressure will depend upon the distensibility of the arteries and this depends, among other things, upon the height of the arterial pressure. In addition to raising the arterial pressure, systolic ejection replaces blood which is lost by drainage through the arterioles. Since the ratio of blood which drains during systole and during diastole is probably not constant, variations in the rate of arteriolar drainage may also disturb any quantitative relation between stroke volume and pulse pressure. Thus while there may be a positive statistical correlation between pulse pressure and stroke volume (see below) it is not of such a high order that the one may be predicted from the other with accuracy in individual cases. Such a prediction must depend upon the evaluation of individual arterial distensibility, knowledge of the pulse pressure in the arterial tree and its several parts and the estimation of arteriolar drainage.

The classical method of evaluating arterial distensibility is from pulse wave velocity (2, 3). In calculating the stroke volume this approach was used by Broemser and Ranke (4) and by Bazett *et al.* (5), to cite two of the schools. The first of these authors represent a group which used the overall pulse wave velocity, while Bazett estimated separately the distensibility of different sorts of arteries from their individual pulse wave velocities.

A critical study of the relation between pulse wave velocity and arterial distensibility has led us into discouragement. The viscosity of the vascular wall brings another and perhaps a variable term into the Moens equation which relates these two variables. There is constant trouble in estimating the diastolic size of the arterial tree. This is an important factor because the pulse wave velocity measures not the absolute distensibility in cubic centimeters but rather its value in relation to the diastolic size. The pulse wave velocity may vary 25 per cent or more with a constant arterial uptake (distensibility in cubic centimeters) if there is a change in the tone of the arterial wall which results in a change of 25 per cent or more of the diastolic capacity.

Now a study of pressure volume curves derived from stretching contracted and

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relaxed arterial rings has led to the conclusion that since the curve from the contracted ring is essentially parallel to that from the relaxed ring (3, 6, 7) a change in the tone of arterial wall is not important in governing the amount of blood stored in the arteries as a result of ejection (uptake). The primary factors seem to be the amount of pressure rise resulting from systole and the pressure range over which this rise takes place. It is to be recognized further that at the moment the semilunar valves close there are widely different pressures obtaining in the various parts of the arterial tree.

To simplify the use of these facts in calculating the stroke volume of dogs we have tabulated certain constants relating pressure, volume, and transmission time. In using these data to measure the stroke volume it is necessary to establish six to ten pressure-time points on a central pressure pulse curve, to read off the corresponding volumes and by a series of simple arithmetical computations to arrive

TABLE 1

*Capacity per square meter body surface of the arterial tree and its several parts at different pressures*

PRESSURE	ARCH	HEAD AND THORACIC	VISCERA AND ABDOMINAL	LEGS	TOTAL
<i>mm. Hg</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
20	2.1	5.1	1.9	1.8	10.9
40	5.0	11.7	4.3	4.0	25.0
60	8.7	19.5	7.1	6.5	41.8
80	12.5	28.2	9.7	8.7	59.1
100	16.2	36.6	11.7	10.7	75.2
120	19.1	42.9	13.7	12.5	88.2
140	21.2	48.1	15.3	14.0	98.6
160	22.9	51.9	16.6	15.2	106.6
180	24.0	54.0	17.5	16.0	111.5
200	24.9	55.8	18.3	16.7	115.7
220	25.7	57.3	19.0	17.3	119.3

at the stroke volume. The method has been tested over wide ranges and found to be applicable to resting dogs.

**METHOD OF COMPILING MEASUREMENTS.** From the data gathered for other publications (3, 6, 7, 8), the distensibility of the various parts of the arterial tree was tabulated. The table (table 1) furnishes figures from which a series of smoothed curves can be drawn on millimeter paper so that uptake in cubic centimeters corresponding to actual pulse pressures can be found. The figures are derived from the average of many volume pressure points through which smooth curves were drawn whose contours agreed with those developed from stretch curves made on aortic and arterial rings. A final revision upward was made proportional to the surface area of the dog (9). The figures in the table are regarded as furnishing a step in calculating the stroke volume per square meter.

From these same data transmission times of the pulse wave through these same parts of the arterial bed were entered in table 2. It will be seen from this

table that these times vary with diastolic pressure. The sixth column in table 2 is a "weighted transmission time for drainage ( $T_w$ ). It is a figure derived from the average time required for the pulse wave to reach the terminal arterioles of each bed, weighted by their relative drainage. In making this average, for example, a smaller weight was given to the long transmission time to the hind legs as compared to the short transmission time to the head, because the drainage through the arterioles of the head and fore legs is much greater than that to the hind legs.

In both tables 1 and 2 the arteries going to the head and fore legs are classified with the thoracic aorta rather than with the arch, their true anatomical sources, because these parts of the arterial tree, having similar transmission times, are distended simultaneously. Likewise, the abdominal aorta and visceral arteries

TABLE 2

*Pulse wave transmission times to the parts of the arterial tree at various diastolic pressures for dog of 15 kgm.*

PRESSURE	ESTIMATED TRANSMISSION TIMES			
	For uptake			For drainage
	Head and thoracic (Th)	Visceral and abdominal (Tv)	Leg (Tl)	Weighted average ( $T_w$ )
<i>mm. Hg</i>	<i>msec.</i>	<i>msec.</i>	<i>msec.</i>	<i>msec.</i>
20	59	110	170	129
40	48	89	136	101
60	39	75	112	84
80	33	64	94	72
100	28	55	81	52
120	24	47	70	53
140	20	40	59	46
160	18	34	50	39
180	16	28	42	33

are treated as a unit. On this basis we work with the arterial tree as though it were composed of four separately distensible systems: 1, the arch; 2, the thoracic aorta, head, fore leg artery system; 3, the abdominal aorta, visceral artery system, and 4, the arterial system to the hind legs and pelvic region. The systolic uptake of the arterial tree is the sum of those of each of the four systems, as calculated from the net pressure change from diastolic level which has occurred in each particular bed when the semilunar valves close.

In some pulses, systole may be over before the pulse wave has reached the leg arteries. Hence, the latter are not regarded as participating in the systolic uptake, for their later distention is due to the redistribution of blood already measured elsewhere. In other pulses the incisura, marking the closure of the semilunar valves, occurs at a pressure as low as diastolic. This is taken to mean that the arch has, at this time, no uptake, and that the blood which had distended the arch earlier in systole is measured by distention elsewhere.

*Taking and measuring the central pressure pulse.* To measure the stroke volume of a dog a record of a central pressure pulse is first made from either the carotid artery or the root of the aorta. The carotid record can be made by exposing the artery low in the neck under local anesthesia. A short, 22 gauge hypodermic needle, connected to the leaden line of the manometer system (10) is thrust into the artery without restricting arterial flow, so that reflected waves from points of occlusion are avoided. After the record is taken, the needle can be removed with but minor bleeding, which can be stopped by compression if necessary. The wound can be closed after dusting with sulfanilamide powder.

The aortic contour is taken only from sacrifice animals under anesthesia. The left carotid is opened as for ordinary cannulation and an 8 inch 16 G. stainless steel sound, attached to the manometer, is pushed down the carotid and worked into the ascending aorta until the semilunar valves are felt. It is then withdrawn an inch. At autopsy the end of the cannula is usually found in the arch of the aorta, at or near the opening of the brachiocephalic artery.

The pressure pulses in the aorta and unoccluded carotid are not significantly different. They should be recorded at a camera speed of approximately 5 cm./sec. to facilitate later measurements. The manometer should then be calibrated against a mercury manometer and a calibration curve plotted so that readings can be made accurate to  $\pm 1$  mm. Hg. As has been pointed out previously (11) measuring the calibration curve and the record itself can best be done by making on a lantern slide a contact print of a 1-10 photographic reduction of a 50 x 60 cm. sheet of millimeter paper. The distances from the bottom and left hand edges of the paper are numbered at each 5 cm. intersection in mirror writing. The squares on the lantern slide are 0.1 mm. across and can be used to measure the time and pressure dimensions of the record with considerable accuracy. The lantern slide may be weighted with a frame of  $\frac{1}{2}$  inch square brass, waxed to the side of the glass and covering up to its edges. It is laid on the record emulsion side down, so that the record and measuring plate may be viewed in the same focus and without parallax by means of the low power of a binocular dissecting microscope.

If the pressure pulses are reasonably constant, any one on the record may be measured. If there is an arrhythmia, a typical one must be selected which is as near as possible the average for systolic and diastolic pressure, and for cycle duration.

Diastolic pressure ( $Pd$ ) (see fig. 1) is then measured and the transmission times for the head system ( $Th$ ), for the visceral system ( $Tv$ ) and for the leg system ( $Tl$ ) are noted from table 2 or preferably from a plot made from the data in the table. The incisura is then found and its pressure noted ( $Pi$ ). Pressure in the head system ( $Ph$ ) at the time of incisura is found by laying  $Th$  back from the incisura into systole and measuring the pressure at this instant. Similarly the pressures obtaining at the end of systole in the visceral ( $Pv$ ) and leg beds ( $Pl$ ) are found using the incisura time,  $Tv$ , and  $Tl$ .

The systolic uptake of the arch is calculated from the difference in the volume of the arch at  $Pd$  and at  $Pi$ . These two volumes are given in table 1 (or better in



a chart plotted from the data in table 1) and their difference recorded as  $U_a$ . Similarly the uptake of the head, visceral and leg beds ( $U_h$ ,  $U_v$  and  $U_l$ ) are derived from the data in table 1 using the difference in the volume at  $P_d$  and  $P_h$ ,  $P_v$  and  $P_l$  respectively. The total arterial uptake ( $U$ ) is the sum of  $U_a$ ,  $U_h$ ,  $U_v$  and  $U_l$ .

The stroke volume is the sum of arterial uptake and arteriolar drainage during systole. Systolic drainage is evaluated according to the principle introduced by Bazett (5). Arteriolar drainage during diastole equals arterial uptake ( $U$ ). The findings of Whittaker and Winton (17) are accepted, so that systolic drainage can

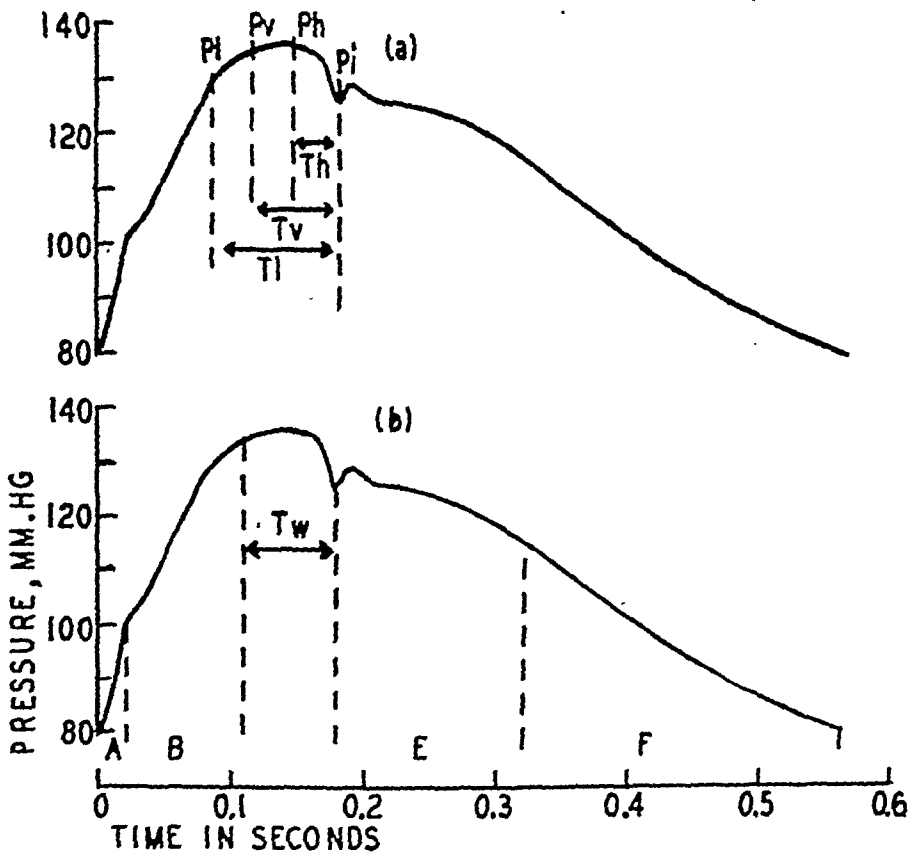


Fig. 1. The subdivision of a central pressure pulse contour for the calculation of the stroke volume of the heart.

be related to diastolic drainage in proportion to the products of the effective pressure (i.e., pressure above 20 mm. Hg) of systole and diastole and their respective durations.

The time of diastole is measured from the incisura to the next systolic upstroke. For our purposes  $T_w$  is to be added in (see below). The effective diastolic pressure is taken as 20 mm. Hg less than the average pressure measurements made at the incisura, at the end of diastole and at two or three intermediate points. The product of the duration of diastole and its effective pressure is referred to as the diastolic drainage area ( $D_a$ ).

The drainage time of systole is measured by counting back from incisura the weighted transmission time for drainage ( $Tw$ ) (table 2). The effective systolic pressure is 20 mm. Hg less than the mean systolic pressure as measured on the pulse curve from the beginning of systole up to the end of the drainage time of systole. The product of the drainage time of systole and the effective systolic pressure is the systolic drainage area ( $Sa$ ).

Systolic drainage ( $Sd$ ) is then given by the relationship

$$Sd = \frac{Sa}{Da} U \dots\dots\dots (1)$$

And the stroke volume ( $SV$ ) by the relationship

$$SV = U + Sd \dots\dots\dots (2)$$

*An example will serve to illustrate the calculation.* From measurements of the pressure pulse given in figure 1, it is found that the diastolic pressure is 80 mm. Hg, systolic pressure 135 mm. Hg, and the pressure at the incisura is 125 mm. Hg.

From table 2 the transmissions to the several beds are as follows:  $Th = 33$  msec.,  $Tv = 64$  msec.,  $Tl = 94$  msec. The pressures are:  $Pi = 125$  mm. Hg,  $Ph = 135$  mm. Hg,  $Pv = 135$  mm. Hg and  $Pl = 130$  mm. Hg (fig. 1a). From table 1 the uptake of the several beds is as follows:  $Ua = 7.2$  cc.,  $Uh = 18.9$  cc.,  $Uv = 5.9$  cc., and  $Ul = 4.6$  cc. The sum of these, the arterial uptake  $U$ , is 35.9 cc.

Diastole can be divided into 2 areas ( $E$  and  $F$ , fig. 1b), whose durations are 0.14 and 0.24 sec. respectively. In addition, an area  $G$ , which is not shown on the figure, must be added. Its duration is the weighted transmission time ( $Tw$ ) and its pressure is diastolic. It represents the diastolic drainage which occurs after ejection begins and before the pulse wave reaches the periphery. It is to be thought of as following area  $F$ . The mean effective pressure values for the three areas are:

$$E = \frac{(128 - 20) + (114 - 20)}{2} = 101 \text{ mm. Hg}$$

$$F = \frac{(114 - 20) + (80 - 20)}{2} = 77 \text{ mm. Hg}$$

$$G = 80 - 20 = 60 \text{ mm. Hg.}$$

The areas will then be:

$$E = 101 \times 0.14 = 14.1$$

$$F = 77 \times 0.24 = 18.5$$

$$G = 60 \times .07 = 4.2$$

$$\text{Sum } (Da) = 36.8$$

The weighted transmission time for drainage is 72 msec. from table 2. Since the length of systole is 180 msec., the net drainage time of systole will be  $180 - 72 = 108$  msec. (fig. 2b).

The systolic drainage curve can also be divided into two areas, A and B, whose mean effective pressure values will be:

$$A - \frac{(80 - 20) + (104 - 20)}{2} = 72 \text{ mm. Hg}$$

$$B - \frac{(104 - 20) + (134 - 20)}{2} = 99 \text{ mm. Hg}$$

Since the times of A and B are 0.03 and 0.08 sec., the areas will be:

$$A - 72 \times 0.03 = 2.2$$

$$B - 99 \times 0.08 = 7.9$$

$$\text{Sum (Sa)} = 10.1$$

Systolic drainage will then be (equation 1)

$$\frac{10.1}{36.8} \times 35.9 \text{ cc.} = 9.9 \text{ cc.,}$$

and from equation 2 the stroke volume will be:

$$35.9 \text{ cc.} + 9.9 \text{ cc.} = 45.8 \text{ cc.}$$

The stroke volume determined by the dye injection technique, taken simultaneously, was 49.2 cc.

*To test the validity of the prediction of stroke volume by this calculation from the pressure pulse contour* a series of 45 experiments was performed on dogs, in which the cardiac output was measured by the dye injection method (12, 13, 14) while the pressure pulse was being recorded either from the root of the aorta, with a sound down the carotid artery, or from a needle in the carotid artery. The records were made optically by means of an adequate manometer (10). To assure that the experiments covered a wide variety of physiological states, diastolic pressure values ranging from 18 to 172 mm. Hg were obtained by means of hemorrhage, by injections of acetylcholine, epinephrine, or ephedrine, and by the use of a dog with renal hypertension (cross on fig. 2), kindly shipped to us by Dr. I. H. Page. These maneuvers, together with variations in the depth and type of anesthesia, caused presumably a large variety of combinations of myocardial activity and vasomotor resistance.

The correlation between the stroke volume as estimated from dye injection and that estimated from the foregoing calculation from the pressure pulse contour is  $r = 0.994$ . The average variation between the two measurements is  $\pm 8.2$  per cent. The range is from + 35 per cent to - 13 per cent. The distribution of the measurements is plotted in figure 2.

Two other ways of calculating the stroke volume from pressure pulse data were tried. The first was by the use of the pulse pressure alone. An empirical equation which relates our dye injection stroke volume to our measured pulse pressures is

$$SV = 0.62 Pp + 2.6.$$

When this equation was used to predict individual dye injection stroke volume from individual pulse pressures the average error was  $\pm 25.8$  per cent and ranged from  $+140$  per cent to  $-45$  per cent. The correlation between pulse pressure was  $r = 0.78$ . This shows that while pulse pressure establishes a general statistical trend in the evaluation of stroke volume the variation in arterial distensibility and in other factors rob the measurement of individual predictive accuracy.

One of the most important and predictable of the changes in arterial distensibility is related to diastolic pressure. Over certain pressure ranges, the higher the diastolic pressure the greater the distensibility. Over other ranges the opposite relation obtains. This relationship is given in table 1, column 6. The

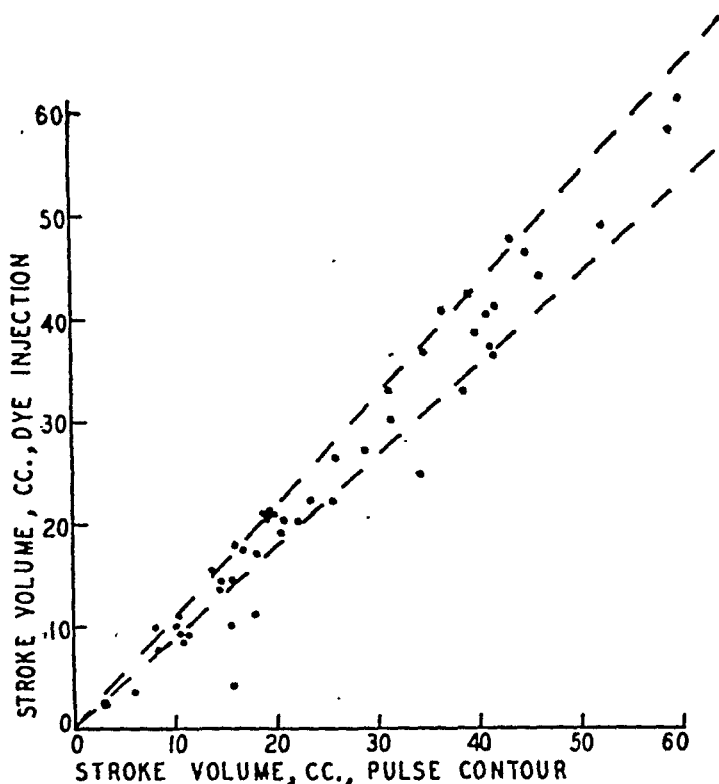


Fig. 2. The relationship between the stroke volume of dogs, as measured by the dye injection technique, and as calculated from simultaneously recorded pressure pulse contours. The dotted lines represent a deviation of  $\pm 10$  per cent from the line of identity.

values represent the summation of measurements of the distensibility of the several parts of the arterial bed. Correcting the pulse pressure values for the distensibility of the arteries at the experimental diastolic pressure gives another set of predictions which can be compared with the dye injection results. The correlation coefficient is 0.88, a distinctly better fit than that obtained with crude pulse pressure measurements. When, however, the equation of best fit is used to predict the stroke volume from the individual corrected pulse pressures, the average error is still  $\pm 22.6$  per cent, ranging from  $+280$  to  $-45$  per cent.

*Errors of the dye injection method.* This method was chosen for comparison with the pressure pulse calculation for several reasons. It is easier to apply than

the Fick method, which is the only other method of equal fundamental soundness. It has been shown to give values equivalent to the Fick method (12). Perhaps most important is the fact that the method is based on the resultant of a large number of individual dye concentration determinations, which must conform to an easily recognizable pattern for the cardiac output figure to be valid. If this pattern is not clear, the determination is doubtful and may be discarded. The overall error of the method is of the order of  $\pm 10$  per cent.

In the data plotted in figure 2, the most questionable fits are in the range of low stroke volumes. In these animals the dye concentration curve failed to descend to low levels before recirculation and hence did not clearly estimate the once circulated dye. We are therefore inclined to consider the calculations from the pressure pulse contour as being more valid than those from the dye method under these particular conditions, because the pattern of the dye curve left us in doubt as to whether it was a good experiment.

*The errors of the pulse pressure calculation.* There are certain unsubstantiated assumptions in this calculation. Whether these introduce systematic error can only be seen in the degree of agreement obtained between the stroke volume calculated from the pressure pulse curve and that calculated from a direct dilution method whose errors could hardly have the same bias. The agreement is mostly within  $\pm 10$  per cent, i.e., within the spread indicated by the dotted lines of figure 2 and extends over at least a six fold range of stroke volume. The possible sources of error may be classified into four sorts.

A. *Estimation of transmission times.* We have used average transmission times, regardless of the size of the dog. If the dog is larger than our average (15 kgm.), true transmission times will be somewhat greater than those used in the calculation. While this will usually increase uptake figures, it also will diminish drainage figures. Hence the net error in our series of animals, ranging in weight between 6 and 22 kgm. is so small as to be covered over by other random fluctuations.

B. *The use of a constant volume-pressure relationship* may be in question because recently (7) stress has been laid on the fact that changes in the tone of arterial smooth muscle causes changes in the diastolic capacity of the arterial tree. This affects the relation between diastolic size and uptake, and hence pulse wave velocity, but it has a much smaller effect upon uptake itself. The volume pressure curves derived from stretching contracted and relaxed arterial rings are essentially parallel, so that the uptake due to a given pulse pressure is the same, within our error, regardless of changes of vascular tone.

C. *The use of a central pulse contour* for the estimation of volume changes in a peripheral bed is questionable because peripheral contours carry reflected waves and may be very different from central contours. The systolic pressure in the lower abdominal aorta and in the legs is known to be considerably in excess of that in the root of the aorta. The arteries to the head and fore legs, since they arise directly from the arch of the aorta, are distended by a central pulse. The main arteries to the viscera, which arise from the lower aorta, may be considered to originate so near the nodal point of the aortic resonating system (11, 15) that

the distending pulse is essentially central in character. Corrections for reflected waves need affect, therefore, only figures for the leg arteries. When the uptake and systolic drainage of the abdominal and leg arteries are calculated from the femoral pulse contour instead of from the central pulse contour, both figures increase. The increase is of the order of 4 per cent and has probably been compensated for by an overestimation of uptake elsewhere, because a 4 per cent upward correction of the pulse wave stroke volume makes the fit with the dye results worse rather than better.

D. *Use of a linear pressure-flow relationship* to calculate systolic drainage can be questioned on the ground that there is good evidence that arteriolar flow is proportional to a power of pressure rather than to pressure itself. Thus Green and his co-workers (16) find that the relationship could best be expressed by:  $F = (P/C)^n$ , where  $C$  and  $n$  vary under different conditions. In their experiments, the exponent  $n$  is usually less than 2. If arteriolar flow is proportional to a power of pressure rather than to pressure itself, the calculation we propose underestimates systolic drainage. The underestimate is greater, the more the average systolic pressure exceeds mean diastolic pressure. But this spread between systolic and diastolic pressure is indicative of vasodilatation. Whittaker and Winton (17) have shown that with complete vasodilatation, pressure and flow have a linear relation and Green *et al.* have indicated that it is only in the presence of vasomotor tone that the relationship becomes exponential.

Several pulse curves have been recalculated on the assumption that the flow is proportional to the square of the pressure. In most cases the difference was negligible, and where it was not, the peripheral resistance was so low that considerable vasodilatation could be assumed, and hence a linear pressure-flow relationship.

#### SUMMARY

The stroke volume of dogs was measured by the dye injection method in 45 experiments displaying a wide variation of physiological conditions. Simultaneous pressure pulse contours were recorded.

A rough correlation ( $r = 0.78$ ) was found with pulse pressure, a slightly better one ( $r = 0.88$ ) was found with pulse pressure corrected for the variation in distensibility which accompanies variations in diastolic pressure and a very good one ( $r = 0.994$ ) was found with a calculation based upon details of the pressure pulse contour, transmission times to the various parts of the arterial tree and the distensibility of those parts.

Tables and formulas are presented from which the stroke volume of dogs can be calculated to agree within  $\pm 8.2$  per cent (average difference) with the stroke volume as measured by dye injection and heart rate.

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# QUANTITATIVE CALCULATION OF THE TIME COURSE OF CARDIAC EJECTION FROM THE PRESSURE PULSE<sup>1</sup>

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It was argued elsewhere (1) that the contour of the cardiac ejection curve could be derived from the amount of blood required for the distention of the successive parts of the aorta by the pulse wave. Since no quantitative evaluation was given to the distensibility of the great branches of the aorta and of their finer ramifications, no quantitative relationship was postulated between the amount of blood ejected in cubic centimeters and the actual pressure change in millimeters of mercury.

After the publication of the qualitative study of the contour of the ejection curve, a series of measurements of the capacity and distensibility of the aortic branches was undertaken, in the hope that there would result data from which the stroke volume and the ejection curve could be quantitatively calculated. In a preceding paper (2) it was shown that stroke volumes calculated from the pressure pulse contour agreed quite closely with those calculated from the dye injection method. Since the stroke volume defines the amplitude of the ejection curve, data are herewith presented from which the quantitative nature of the ejection can be determined throughout its duration, from the central pressure pulse contour, and from the capacity and distensibility of the various parts of the arterial tree.

As a fundamental guide in apportioning the cardiac ejection to the various parts of the arterial tree the capacities of these parts were measured in the following manner.

A 14 kgm. dog, freshly killed, was perfused, via the carotid artery, with several liters of physiological saline to flush potential clots from the arterial bed. A suspension of Portland cement and white clover seeds (diameter ca. 1 mm.) in 20 per cent formaldehyde was injected rapidly under a pressure of 100 mm. Hg. The carotid was tied, holding the mass under pressure. The carcass was then preserved in formaldehyde. After two days, when the cement was well set, the body was cut into segments; and x-ray pictures were made of each segment (the cement being opaque). Using these films as guides, the arteries were dissected free, and their lengths and diameters recorded. As judged by a comparison of vessel size in a living dog of the same weight, the diameters actually did correspond to the intended pressure level of 100 mm. Hg.

The volumes estimated from the measured diameters are given in table 1. Arteries smaller than 1 mm. in diameter were not injected, and were therefore not measured. The rest were grouped into three large categories, depending upon diameters. The vessel systems themselves were then arbitrarily grouped

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according to the region of exit from the aorta. For example, the "head" category included all vessels arising from the ascending aorta and arch, and therefore included not only vessels to neck and skull, but the coronary and superior epigastric arteries, and the arteries of the forelegs. Reference to table 1 shows that some 44 per cent of the entire arterial volume is contained in this vessel system. Moreover, if the aorta itself be excluded, then the vessels of the head

TABLE 1

*Arterial dimensions at 100 mm. Hg in a 14 kgm. dog injected with cement*

VESSEL SYSTEM	LARGE (3-10 MM. DIAM.)			MEDIUM (2-3 MM. DIAM.)			SMALL (1-2 MM. DIAM.)			TOTAL VOL.	% OF TOTAL ARTE- RIAL VOL.
	No.	Total length	Vol.	No.	Total length	Vol.	No.	Total length	Vol.		
		cm.	cc.		cm.	cc.		cm.	cc.	cc.	
Aorta.....	1	49	38.6							38.6	23.3
Common carotid.....	2	48	13.7	10	12	0.6	21	107	2.0	16.3	9.8
External carotid.....	2	2	0.2	12	5	0.3	28	80	1.8	2.3	1.4
Internal carotid.....	2	12	0.8	8	8	0.4	20	80	1.9	3.1	1.9
Vertebral.....	2	45	9.7	24	17	1.0	40	250	4.4	15.1	9.2
Costocervical.....	2	32	2.4	2	10	0.3	4	10	0.2	2.9	1.7
Coronary.....	5	15	1.1	20	64	2.8	20	41	0.3	4.2	2.5
Sup. epigastric.....				24	38	1.8	47	164	3.3	5.1	3.1
Brachials.....	8	43	15.8	18	122	4.5	60	200	3.4	23.7	14.3
Total "head".....	23	197	43.7	118	176	11.7	240	932	17.3	72.7	43.9
Intercostals.....							46	96	1.8	1.8	1.1
Coeliac.....	4	11	1.1	14	38	1.8	18	50	1.1	4.0	2.4
Sup. mesenteric.....	1	8	2.2	22	80	3.6	30	85	1.6	7.4	4.5
Renals.....				4	5	1.2	4	8	0.7	1.9	1.1
Segmentals.....							25	175	5.9	5.9	3.6
Total "viscera".....	5	19	3.3	40	123	6.6	123	414	11.1	21.0	12.7
Ext. iliac.....	2	3	0.6	6	60	2.8	14	240	4.6	8.0	4.8
Femorals.....	6	43	13.7	10	79	4.6	34	400	7.0	25.3	15.3
Total "legs".....	8	46	14.3	16	139	7.4	48	640	11.6	33.3	20.1
Total "body".....	37	311	99.9	174	538	25.7	411	1,986	40.0	165.6	

and arms contain 56 per cent of the extra-aortic arterial volume. Vessels arising from the descending aorta, exclusive of the terminal bifurcation of the aorta into iliacs, have 17 per cent of the total extra-aortic volume; the iliacs, 27 per cent.

Now the ascending aorta and arch, hereafter called simply the "arch", again at 100 mm. Hg pressure, represent 40 per cent of the total aortic volume, despite the relatively short length.

It was observed by Hales in 1733 that the branches of an artery have a larger aggregated cross area than the vessels from which they spring. This seems to be true at all pressures in peripheral branchings but is only true at low pressures in case of the ascending aorta and its branches. At higher (physiological) pressures the ascending aorta is so distended that its cross area is greater than that of the combined brachiocephalic, left subclavian and descending aorta. This fact is descriptive of the great reservoir function of the ascending aorta needed, perhaps, to sustain the large outflow through the arteries to the head and fore-legs.

To evaluate the contribution of the separate parts of the arterial system to the total uptake, the aortas of four freshly killed dogs were cannulated with a large cannula passed through the left ventricle and tied into the ascending aorta. The arterial tree was cleared of blood by saline perfusion. The segmental arteries were tied off, and the arteries to the head, viscera and legs were clamped with hemostats. Varying known volumes of saline were injected suddenly into

TABLE 2

*Volumes per square meter body surface of the various parts of the arterial tree at various pressures*

PRESSURE	ARCH	THORACIC	ABDOMINAL	HEAD	VISCERA	LEGS
<i>mm. Hg</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
0	8.3	13.9	7.2	99.3	28.8	46.3
20	10.5	15.1	8.0	103.0	30.0	48.0
40	13.3	16.7	9.2	107.8	31.2	50.3
60	17.0	18.9	10.4	112.6	32.7	52.5
80	20.8	21.9	11.7	118.1	34.1	55.0
100	24.5	25.1	12.7	122.0	35.1	56.8
120	27.3	27.9	13.5	125.9	36.1	58.6
140	29.5	30.6	14.4	129.6	37.0	60.3
160	31.1	33.0	14.9	132.0	37.8	61.5
180	32.3	34.4	15.4	133.9	38.3	62.3
200	33.3	35.4	15.8	135.5	38.7	62.9

the aorta from a burette connected at the bottom with the cannula and at the top with the air pressure. Pressure changes were recorded optically during filling and drainage, and the volume pressure relations of the aorta were calculated as indicated elsewhere (2). After opening the proper hemostats, this procedure was repeated for the aorta plus the head arteries, the aorta plus the visceral arteries and the aorta plus the leg arteries. Thus the distensibilities of the several beds was evaluated.

In order to use these distensibility data in the construction of ejection curves, the volumes of the several parts of the aortic tree were tabulated (table 2) at measured pressures. Actual measurements from the above described saline injection experiments were supplemented by data derived from stretching aortic and arterial rings and calculating volume pressure relations. Before tabulating, the figures were multiplied by the ratio of one square meter to the surface area of the dog.

A third table (table 3) was constructed from numerous measurements of transmission times of the pulse wave from the root of the aorta along the aorta and to the periphery.

The construction of the arterial uptake curve is carried out in essentially the same fashion as is the construction of the aortic uptake curve described elsewhere (1). The arterial tabulations are more extensive than the aortic in that six arterial subdivisions must be considered instead of the three aortic subdivisions. These six subdivisions are: 1, arch; 2, "head" arteries; 3, thoracic aorta; 4, abdominal aorta; 5, visceral arteries, and 6, leg arteries. The principles of the computation are the same as those given in table 3 of an earlier publication (1). Time of systole is divided into 10 msec. intervals and the average pressure read for each interval. Reference to table 2 indicates the amount by which the whole of the arch is distended by the difference between diastolic pressure and the pressure obtaining during the first 10 msec. of systole. Now if the pulse wave

TABLE 3

*Transmission times through the various parts of the arterial bed of a 15 kgm. dog*

Transmission times to these various parts can be derived from this table. Thus transmission time to the leg arteries equals the sum of transmission times through the arch, thoracic and abdominal aorta; to the visceral arteries equals the sum of times through arch and thoracic aorta, etc.

PRESSURE	ARCH	THORACIC	ABDOMINAL	HEAD	VISCERA	LEGS	T <sub>w</sub>
mm. Hg	msec.	msec.	msec.	msec.	msec.	msec.	msec.
20	18	58	34	64	33	84	129
40	15	46	30	51	29	58	101
60	12	38	26	42	27	48	84
80	10	31	22	36	26	42	72
100	9	25	19	29	24	36	62
120	8	22	18	24	18	30	53
140	7	18	16	19	16	24	46
160	6	16	14	18	12	20	29
180	5	13	11	17	10	16	33

is transmitted through the arch in 10 msec., all of this distention is assigned to the first 10 msec. segment of the arch. If, on the other hand, diastolic pressure is such that transmission over the arch requires 15 msec.,  $\frac{2}{3}$  of the arch uptake as read from table 2 is assigned to the first 10 msec. and  $\frac{1}{3}$  to the second. During the second 10 msec. interval the pulse wave is distending simultaneously the thoracic aorta and the "head" arteries, (i.e., those of the head, shoulders, and fore-legs). The uptake of these parts as read from table 2 is partitioned among the succeeding 10 msec. segments in proportion to the transmission time through them (table 3). Distention of the visceral arteries, abdominal aorta, and leg arteries is treated in the same manner and added in at the proper time as indicated by the transmission times to and through these parts of the arterial tree.

When the table is completed it consists of at least as many vertical columns as there are 10 msec. intervals in the duration of systole. These are partitioned among the six parts of the arterial tree. There is also a horizontal row of figures,

for each systolic 10 msec. interval. These horizontal rows are summated to give the arterial uptake during each 10 msec. interval. Plotted against time, they give the arterial uptake curve.

Assuming a diastolic pressure of 70 mm. Hg, the first 10 msec. horizontal line will contain figures only from the arch. The second horizontal line will contain 2 figures which indicate the uptake of the arch, and 1 each for the thoracic aorta and head arteries. The third will contain 2 figures for the arch, thoracic and head arteries. The fourth line will contain one additional figure for the head arteries and one for the thoracic aorta. During the fifth 10 msec. interval the pulse wave invades the abdominal and visceral arteries and their contribution to the arterial uptake is then to be added in. Further pressure increases and, toward the end of systole, pressure decreases, alter the net uptake of the proximal parts of the arterial tree. This will be recorded in the lower figures of the first few columns. The invasion of the leg arteries by the pulse wave is the last addition to the distended parts of the arterial tree and this is indicated by the establishment of appropriate columns.

Figure 1b is a plot of the time course of distention of each of the several beds. They are summated in figure 1d, UP, which is the arterial uptake curve for the pulse figured above (fig. 1a). The curves were plotted from a table developed as described above.

*Arteriolar drainage.* In our previous derivation of the contour of the ejection curve from the uptake of successive segments of the aorta (1) we assumed that the uptake of the aortic branches could be treated as drainage. This treatment gave an amplitude of ejection (stroke volume) that is less than 60 per cent of the measured stroke volume. Using the estimated uptake of the arterial branches as described above brings the amplitude of the injection curve (stroke volume) into agreement with that measured by dye injection (2), but necessitates treating drainage in a different manner. Systolic drainage should be considered to begin when the pulse wave gets to the periphery rather than at the very beginning of cardiac systole. It should be considered as ending at the time of the closure of the aortic valves, because all drainage after this time is at the expense of blood already accounted for and is therefore properly diastolic drainage.

To determine the partition of drainage in the various arterial beds, we consulted our own measurements (see above), and the literature (3). We assigned 43 per cent of the total drainage to the head arteries, 43 per cent to the visceral arteries and 14 per cent to the arteries of the hind legs and pelvic regions.

The volume of blood ejected distends the arterial tree and raises its pressure. In addition to this, it replaces all blood lost through the arterioles by drainage. To compute the ejection curve, drainage loss must be added to the uptake volume for each of the 10 msec. intervals during which the pulse wave distends the respective peripheral beds ( $Hd$ ,  $Vi$  and  $Le$ ).

On the assumption that arteriolar flow is proportional to pressure — 20 mm. Hg (4) the drainage from each peripheral bed ( $D_F$ ) can be evaluated as a fraction of the arterial uptake  $U$  by means of the following formulation:

$$D_F = \frac{FT_s (P_s - 20)}{Td (Pd - 20)} U$$

in which  $F$  is the fraction that apportions drainage to the three beds (0.14, 0.43, 0.43),  $T_s$  is an interval of time during systole (here 10 msec.) and  $P_s$  is the mean

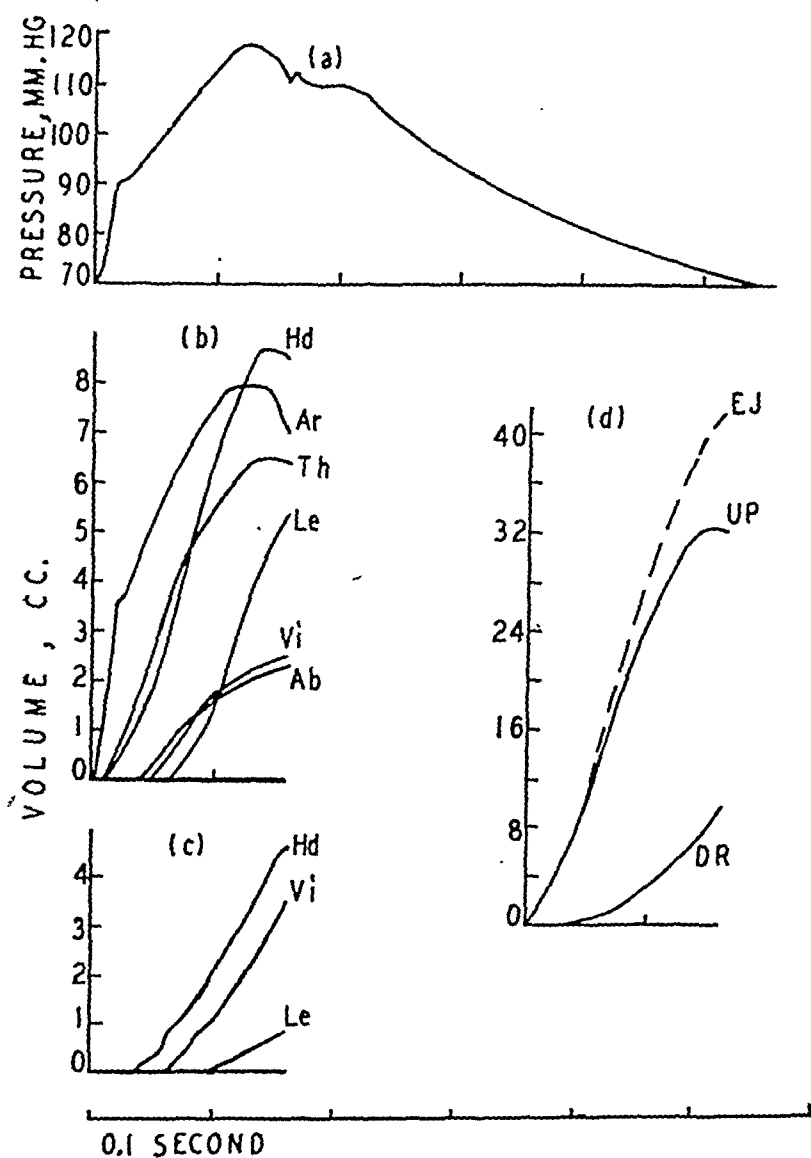


Fig. 1. The calculation of the cardiac ejection curve from arterial uptake.

(a) Typical pressure pulse from central artery.

(b) Uptake resulting from the pressure changes in (a), calculated per square meter for the divisions of the aorta: arch (*Ar*), thoracic aorta (*Th*) and abdominal aorta (*Abd*), and for the distributing arteries to the head and fore quarters (*Hd*), to the viscera (*Vi*), and to the hind quarters (*Le*).

(c) Drainage through the arterioles to the head and fore quarters (*Hd*), viscera (*Vi*), and hind quarters (*Le*).

(d) *DR*, summation of the drainage curves in figure 1c, *UP*, summation of uptake curves in figure 1b, *EJ*, total ejection curve, which is the summation of *UP* and *DR*.

pressure during the same interval.  $T_d$  is the duration of diastole +  $T_w$  (see below) and  $P_d$  is the mean diastolic pressure. Drainage during diastole equals arterial uptake ( $U$ ).

Drainage during systole is considered to begin when the pulse wave reaches the terminal arterioles. Up to that time drainage is diastolic, establishing as it does the pressure just preceding the upstroke of the pulse curve. To determine the time of arrival of the pulse wave at the arterioles the weighted average transmission ( $Tw$ ) time was computed and tabulated (col. 8—table 3). These figures were computed from the transmission times through the three main arterial beds (table 3—cols. 5, 6, 7) and the relative drainage through each.

We have assumed that drainage from each bed begins when the pulse wave has invaded one-third of the bed as measured by the transmission time to and through the bed, and that after this time, systolic drainage increases in proportion as the pulse wave traverses the bed, and after invasion is complete, is maintained proportional to pressure until the semilunar valves close.

On this basis a table was constructed giving drainage figures for each of the beds for 10 msec. intervals, from the pulse curve in figure 1. The table is too complex to publish but is summarized in the drainage curves which are given in figure 1c, summated for each bed at 10 msec. intervals.

RESULTS. Ejection curves have been plotted for several typical pressure pulses (see fig. 2). Simultaneous measurements were made of the stroke volume by the dye method and found to agree with the calculated amplitude of the ejection curve (2). The ejection curve as calculated from the arterial uptake differs significantly from that calculated from the aortic uptake in that it measures up to the true stroke volume and in that it indicates a more prolonged ejection phase.

As in the previous paper (1), we have constructed an ejection curve for a pressure pulse recorded by Wiggers and Katz (5) from an open chest dog. It should be recalled that the pressure pulse was given arbitrary pressure values, and the length of systole is much longer than would obtain in a closed chest animal. Hence the quantitation of neither the ejection curve previously derived from aortic uptake, nor that derived here from the uptake of the whole arterial bed, has a quantitative validity. If the total stroke volume reached by the two ejection curves and by the Wiggers-Katz cardiometer curve, is taken to be the same, then the curve derived from arterial uptake shows closer agreement with the cardiometer curve than does that derived from aortic uptake (fig. 2a).

Cardiac ejection begins slowly and gradually quickens. The curve is at this time derived from the uptake of the proximal parts of the arterial bed. Then the ejection curve becomes straight for a variable period of time, during which it is derived from the uptake of the major portion of the arterial tree and drainage. Toward the end of systole, drainage accounts for an even larger part of the ejection. This is illustrated by figures 2 (b), (c) and (d) which were chosen from our records to exemplify similar diastolic and pulse pressures, with differing peripheral resistance. Figure (c) is from an experiment with a relatively high peripheral resistance and small systolic drainage; (b) is intermediate, while the dog giving (d) shows a low peripheral resistance and large systolic drainage. Curves *c* and *d* seen in figure (e) show the same total stroke

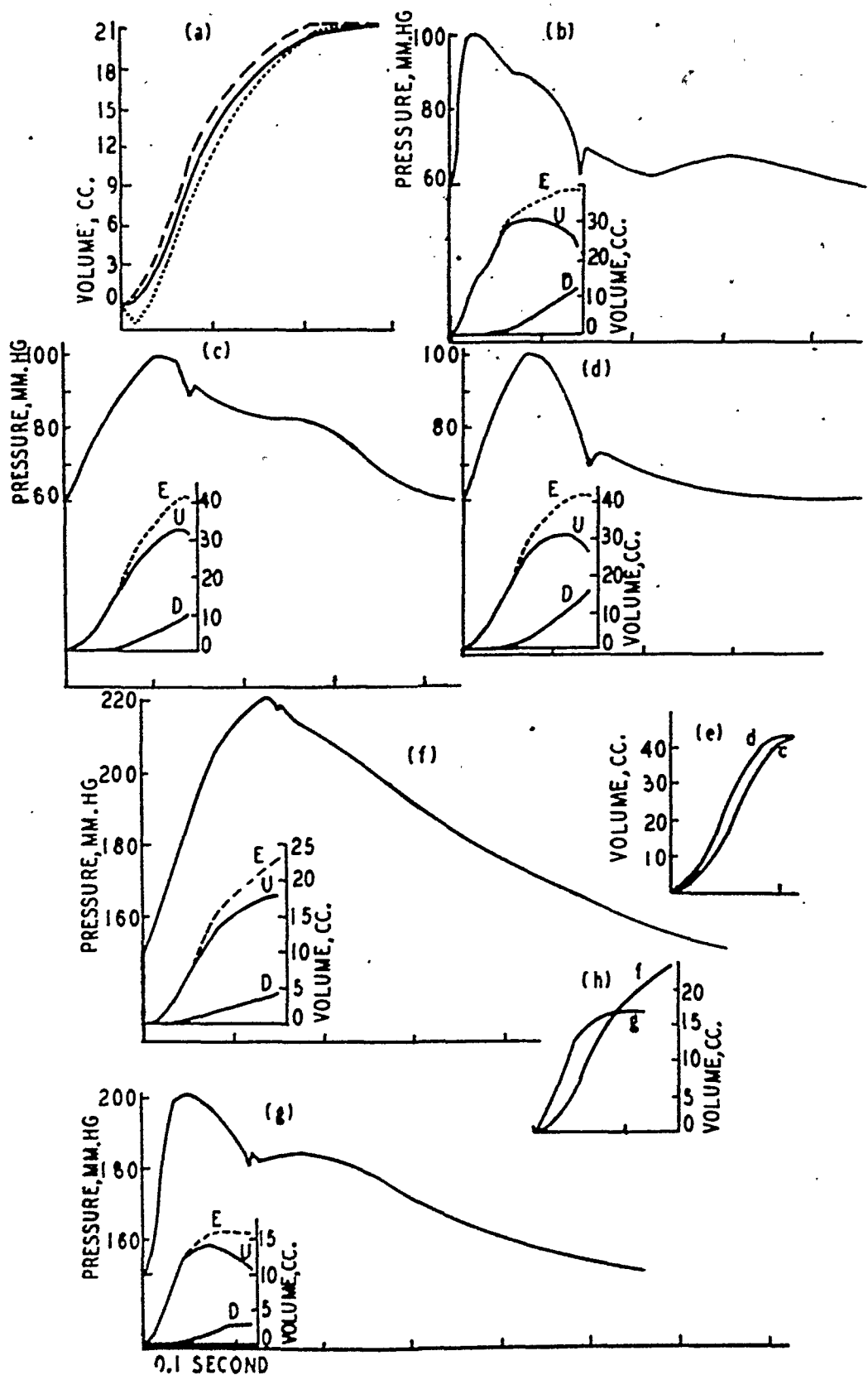


Fig. 2. Typical cardiac ejection curves.

(a) Ejection curve from Wiggers and Katz (5). Dotted line the cardiometer curve by the original authors; broken line ejection curve plotted from the pressure pulse curve (5) by means of aortic uptake; solid line ejection curve calculated by means of arterial uptake.

(b-h) Partition of uptake and drainage to give ejection curves in pulses where the peripheral resistance is low (d), intermediate (b), and high (c, f and g).

volume, and vary only by a small difference in timing. They are made up, however, of quite different components. In *c* uptake is continuous throughout systole, and is supplemented by a small drainage. In *d*, however, uptake is rapid only during early systole. In late systole, the distention even declines. Drainage is sufficient, however, to make the calculation indicate a continuous ejection throughout systole.

In the last two figures ((f) and (g)) the peripheral resistance is high in both experiments and systolic drainage is low. Figure (f) is from a dog with renal hypertension, kindly furnished us by Dr. I. H. Page. Ejection is slow and relatively uniform throughout systole. The hypertension was of long duration and the myocardium was contracting steadily and continuously throughout systole. Figure 2 (g) shows a pressure pulse from a dog responding to a large dose of epinephrine. Ejection was rapid during early systole but was not maintained throughout the period. In these two curves, systolic drainage being similar, any change produced by differences in the ability of the myocardium to sustain ejection is shown directly in the pressure pulse curve. Figure 2 (b) is a similar example of early myocardial effort occurring at a relatively low pressure range.

DISCUSSION. There are certain basic assumptions whose validity is questionable. Most of these affect the quantitative amplitude of the ejection curve (stroke volume). They are 1, use of standard transmission times regardless of dog size; 2, use of standard volume pressure relationship; 3, use of central pulse contour, and 4, use of linear pressure flow relationship for the calculation of drainage. Reasons are given (2) for thinking that these play a small rôle in affecting the amplitude of the ejection curve. It is probable that they also would play a small rôle in affecting its contour.

A marked change in the distribution of drainage between the three main beds can well affect the contour of the ejection curve. Drainage through the arterioles of the head and shoulders occurs early in the cycle and that through the hind legs late in the cycle. For this reason appropriate adjustments in the partition of drainage will have to be made if the method is to be used to calculate the ejection curve under conditions that differ markedly from resting.

A painstaking quantitative reconstruction of the contour of the ejection curve is a necessary prerequisite for calculating the impacts and recoils of the blood in the cardiovascular system. Such calculation is necessary if we may evaluate the ballistocardiogram as a record of such impacts. The ejection curve is also necessary if we are to estimate with any quantitative accuracy the velocities generated in the aorta and consequently the cardiac work done in accelerating blood to these velocities.

There are gross changes in the contour of the ejection curve as an animal deteriorates on the operating table. There are probably similar changes resulting from pathological cardiac processes. These may produce definite changes in the pressure pulse contour. How meticulous a calculation is necessary to evaluate these changes in the ejection curve remains to be seen. Several short cuts have been tried and may prove useful.



## SUMMARY

Detailed measurements of capacity, distensibility and pulse wave transmission time in the dog's arterial tree are presented. Using these data a quantitatively accurate evaluation of the cardiac ejection curve can be computed from the pressure pulse contour.

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COMPARISON OF THE TIME CONCENTRATION CURVES IN  
ARTERIAL BLOOD OF DIFFUSIBLE AND NON-DIFFUSIBLE SUB-  
STANCES WHEN INJECTED AT A CONSTANT RATE AND WHEN  
INJECTED INSTANTANEOUSLY<sup>1</sup>

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The measurement of the cardiac output by the determination of the degree by which a known amount of injected substance is diluted in its course through the heart and lungs, has been carried out according to three different plans. Two of these plans were proposed by Stewart (1, 2) and are based on the assumption that none of the injected material has had time to recirculate through the systemic bed, before the sampling procedure is complete. The third procedure (3, 4) quantitatively evaluates the time of appearance, and the amount of recirculated substance, so that only the once circulated material is used in figuring the cardiac output.

Stewart's first method is based upon the injection of a foreign substance into the blood stream at a constant rate. After a few seconds it is said that the foreign substance reaches a constant concentration in the arterial blood indicating that it has been diluted quantitatively by the aortic stream. This is said to occur before recirculation and to establish a "concentration plateau" from which the cardiac output can be calculated. The calculation may be illustrated by assuming that foreign substance is injected at a rate of 100 mgm. per minute and that the height of the "concentration plateau" is 20 mgm./l. This means that the foreign substance is diluted by a stream flowing at the rate of 5 l. per minute. The formulation may be expressed as

$$f = \frac{i}{c} \dots\dots\dots I$$

where  $f$  and  $i$  are the rates of flow and of injection and  $c$  is the height of the concentration plateau.

Stewart's second method is really a technical simplification of the first. The injection is made in known amount instead of at a known rate, and the time required for the collection of a sample which represents the average concentration of the injected substance in the arterial stream, is used in the calculation. If the rate of sampling is constant, the flow ( $f$ ) is equal to the amount of the injection ( $I$ ) divided by the average concentration ( $c$ ) times the duration ( $t$ ) of sampling.

$$f = \frac{I}{ct} \dots\dots\dots II$$

<sup>1</sup>This investigation was aided by a grant from the American Medical Association.

Thus if 100 mgm. are injected and a 12 second sample shows a concentration of 100 mgm./l. the flow would again be  $1/12$  l. per sec. or 5 l. per minute.

Stewart's calculations do not take into account the possibility that the injected substance may be recirculated so quickly that the sample includes twice circulated as well as once circulated substance. That this possibility is indeed a certainty can be concluded from experiments in which a concentration curve was plotted from successive samples of arterial blood after an instantaneous injection of dye (3, 4) (see also fig. 4 below). When an injection is made into the left ventricle (5, 6) it is practically cleared from the stream before recirculation. When, however, an injection is made into the right heart, or into a vein, such a complete clearance is not seen. For this reason it was found necessary to develop a graphic procedure to separate once circulated dye from twice circulated dye so that the former could be used in calculating the cardiac output with equation II (3, 4).

Attempts have recently been made to measure the cardiac output by injecting a foreign substance at a constant rate, and figuring the flow from the height of a supposed concentration plateau by means of equation I (7, 8). From the evidence given above it is difficult to believe that such a plateau can come about before recirculation is established. Moreover, evidence in the literature of finding such a plateau is tenuous and unconvincing.

Nine experiments were therefore performed in which Brilliant Vital Red was injected into the jugular vein at a constant rate by a motor driven syringe. Successive samples were taken every second by means of femoral puncture and a sampling technique already described (3). The dye was determined after precipitation of plasma proteins by alcohol as described elsewhere (3, 9). Typical concentration curves are given in figure 1. After the dye first appears its concentration increases continuously with no evidence of a concentration plateau. If there is no plateau it would seem clear that the blood flow cannot be calculated by Stewart's first method.

To satisfy ourselves that a true plateau is not even theoretically possible, we had recourse to experiments on models. It has been shown that when dye is injected into water perfused through a glass bulb filled with beads the resultant dye concentration curve is essentially similar to that occurring when an injection of dye is made into blood perfused through the heart and lungs (4).

When dye is injected at a constant rate into a stream flowing through such a bulb, the concentration of successive samples (fig. 2) increases, gradually approaching the concentration predicted from the known rate of injection and flow. Above is the contour of a concentration curve which followed a dye injection made as quickly as possible. When a successive series of such contours is summated, the summated curve can reach a plateau only as the quick injection contour returns to the base line, i.e., as the first injected dye is washed out of the system. Moreover the experimental points (·) show agreement with the summated contour when plotted on the same scale.

In this experiment no recirculation was allowed. In this case, then, a "concentration plateau" theoretically could not, and experimentally did not begin

until the concentration of dye from the initial part of the constant injection had subsided to a negligible value. In the experiment illustrated such a plateau was reached only after six times the initial appearance time had elapsed. Inspection of a large series of dye concentration curves from human and dog experiments has led to the conclusion that the slowest moving dye completes its first circulation only after the lapse of three to ten times the initial appearance time and well after the reappearance time. This in turn means that the "concentration plateau" as defined above can not exist in experiments in which the blood recirculates and dye passes through the lungs.

The injection experiment may be carried out in such a way that the experimental results simulate a plateau. To illustrate this, the concentration curve resulting from an instantaneous injection into a dog is plotted in figure 3, *a*.

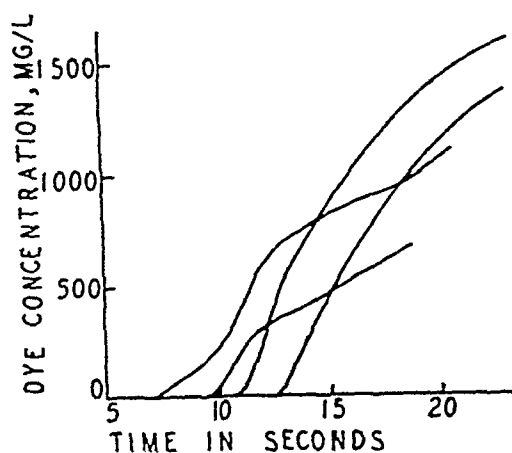


Fig. 1

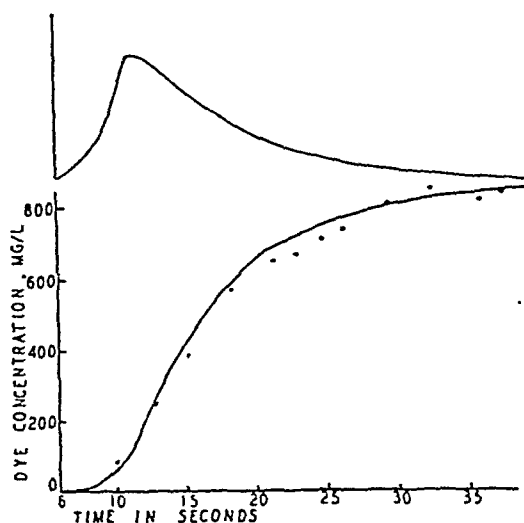


Fig. 2

Fig. 1. Concentration of dye in arterial blood during constant injection, beginning at 0 sec.

Fig. 2. Upper curve: Concentration of dye in model following instantaneous injection. Lower curve: Summation of successive upper curves. Dots .... = Concentration of dye in model following constant injection.

Successive repetitions of this contour are summated so as to give the line *b*, figure 3. This line is similar to those shown in figure 1 and may be taken to represent the concentration curve which would have been given by this dog had the injection been constant instead of instantaneous. The curve *c* is the result of stopping the summation at 13 sec. instead of continuing it indefinitely as in *b*, and may be taken to represent the concentration curve which would have been given by this dog, had injection been continued constant for 13 sec. and then stopped. This curve is gently rounded and begins to fall off about 20 sec. after the start of the curve. Since the upper curve shows that recirculation begins 8 sec. after the start of the curve the summit of curve *c* represents both once and twice circulated blood. Now this curve, *c*, which is derived from successive instantaneous curves is very similar to an experimental curve which

resulted from a continuous injection lasting 13 sec. Such a curve, shown by the points ( $\cdot$ ) is a replotting of Holt's figure 2 (8). It seems to us that the summit of this curve in no way fits the meaning of a "concentration plateau" from which the cardiac output can be calculated.

Various substances have been used to measure the cardiac output by the injection method. Among these are diffusible substances such as sodium chloride, sodium iodide, phenoltetraiodophthalein, and sodium thiocyanate. Contrasting with these are such non-diffusible substances as blood volume dyes.

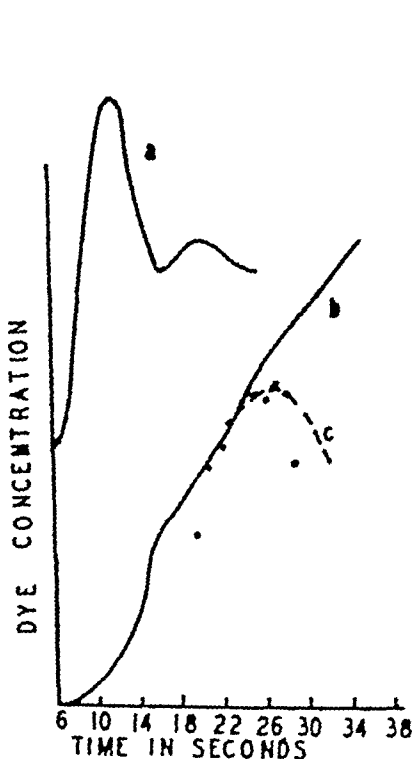


Fig. 3

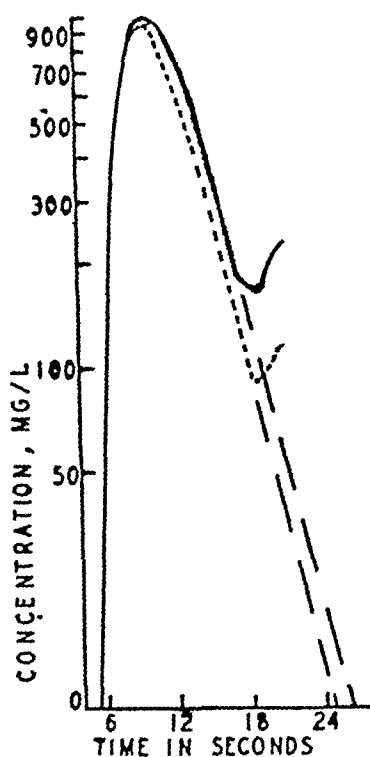


Fig. 4

Fig. 3. a) Concentration of dye in arterial blood following instantaneous injection into dog. b) Summation of successive upper curves, infinite series. c) Same, 13 sec. series. Dots .... = Data from Holt (8).

Fig. 4. Solid line, concentration of dye in arterial blood after instantaneous injection. Dotted line, concentration of thiocyanate after injection of same amount.

Diffusible substances may produce osmotic effects and remain behind in the lungs during the first circulation. Substances that can be used in blood volume determination should be without these handicaps in measuring the circulation rate.

In order to find out to what degree the use of a diffusible substance is inferior to the use of a blood volume dye, injections of a mixture of equal quantities of Brilliant Vital Red and of sodium thiocyanate were made into 5 dogs. The concentration curves of the two substances, given in figure 4, are typical. The thiocyanate curve is lower than the dye curve and the total amount of the salt

recovered on the first circulation is less than that of the dye. In our experiments the cardiac output averages 11 per cent higher when calculated from an injected salt than when determined from parallel dye injections. This difference is regarded as an error on the part of the determination with the sodium thiocyanate. It is similar to the error found with sodium tetraiodophthalein (4), a diffusible dye. Whether it is constant and can be corrected for cannot as yet be determined.

#### SUMMARY

When a foreign substance is injected into a vein at a constant rate, it begins to recirculate before a concentration plateau is established. The cardiac output therefore cannot be calculated from the results of an experiment of this type.

Diffusible substances such as sodium thiocyanate do not pass quantitatively through the heart and lungs on their first circulation as do the blood volume dyes. Such substances should be used with reservation in calculating the cardiac output by the injection method

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# THE CHOLINESTERASE AND ACETYLCHOLINE CONTENT OF THE CHICK RETINA, WITH ESPECIAL REFERENCE TO FUNCTIONAL ACTIVITY AS INDICATED BY THE PUPILLARY CONSTRICTOR REFLEX

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It is now generally accepted among neurophysiologists that acetylcholine and cholinesterase assume a rather specific rôle in the functional activity of the nervous system. Numerous papers have appeared in the literature which bear upon the quantitative significance of both cholinesterase and acetylcholine as they concern the activity of neural structures. The subject has been extensively reviewed by Nachmansohn (1945) and by Feldberg (1945) and needs no elaboration at this time.

The study to be reported in this paper is an analysis of the acetylcholine content and cholinesterase activity of the developing chick retina. An attempt is made to determine if a possible quantitative relationship exists between cholinesterase and acetylcholine content and functional activity in sensory tissues. The retina was selected for this study because of its rather unique neural structure. In the first place, its pattern of development can be divided rather sharply into definite stages of cellular growth and differentiation; secondly, it is a sensory tissue associated with several specific reflexes, and thirdly, most of the cells composing the retina are generally classified as peripheral sensory neurons.

Weysee and Burgess (1906) described three well defined periods of growth in the developing chick retina. First, the period of cell multiplication which extends from the second to the eighth day of incubation; second, the period of cellular re-adjustment from the eighth to the tenth day; and third, the period of final differentiation which takes place from the tenth day to the end of incubation.

These observations are of interest in the present study because they describe the maturation of the various neural units in the chick retina as a process in which most of the cells reach complete differentiation in a relatively limited period of time. This would imply also that the formation of synapses between the various cells takes place within an even shorter period.

Observations by Nachmansohn (1939, 1940) on chick embryo brains support the claim that cholinesterase concentration increases rapidly for a period just prior to hatching and at a time when synapses begin to function. Although this interpretation may be correct, his experimental evidence does not entirely justify this conclusion, since no attempt was made to establish exactly when synapses become functional in the brain. It is doubtful that there is a period in the development of brain tissue when the majority of the synapses become functional at about the same time.

The retina, however, seems to be a neural tissue where the formation of func-

tional synapses might be limited to a short time interval. Consequently, experiments were designed to measure not only the cholinesterase activity and acetylcholine content of the chick retina at various stages of differentiation, but also to determine, if possible, the time when the synapses begin to function.

Furthermore, if it can be assumed that the cells in the retina are peripheral sensory neurons, then this tissue should be valuable material for obtaining evidence concerning the possible rôle of acetylcholine and cholinesterase in the transmission of impulses between sensory neurons and neurons located in the central nervous system.

**MATERIAL AND METHODS.** The chick embryos used in this study were from eggs of a pure strain of New Hampshire red chickens. The eggs were collected twice daily and kept in a cool room until they were ready to be placed in the incubator. In no case were eggs incubated which had been laid more than 24 hours previously. The incubator was maintained at  $37.5^{\circ}\text{C}.$ , and at this temperature the eggs hatched in between 21 and 22 days.

The preparation of the retina for the analysis of cholinesterase was as follows: Embryos of the desired age were taken from the shell and decapitated. The head was placed in a petri dish containing a glycine-NaOH buffer solution. The eyelids and cornea were dissected away and the jelly-like aqueous humor was removed. This exposed the retina which was lifted from the eye with a forceps. After blotting and weighing, the tissue was transferred to small test tubes containing 0.5 ml. of glycine-NaOH buffer mixture (pH 8.4) (prepared according to Tahmisian (1943)) and 500 mgm. of fine white sterile sand was added. The tissue was ground in a glass mortar until it formed a cell free suspension. The mixture was then further diluted with the buffer solution until the final concentration of each sample contained the equivalent of 10 mgm. of tissue per milliliter of suspension.

The tubes were placed in a water bath and kept at a constant temperature of  $37.5 \pm 0.02^{\circ}\text{C}.$  for 2 hours. The milky looking suspension thus obtained constituted the enzyme extract.

The method employed to measure cholinesterase activity was essentially the same as described in a previous study on brain tissue (Lindeman, 1945) and was computed as the acid equivalent (ml. 0.01 N HCl) produced by enzymatic hydrolysis per milligram of fresh tissue per hour.

The acetylcholine in the retina was determined by the following method modified from Feldberg (1943). The retinas from 2 or 3 embryos of the same stage of incubation were homogenized in 0.5 ml. of a chilled extraction solution made up as follows: 10 ml. of Phosphate Locke's solution, 5 ml. of 0.02 M. Phosphate buffer mixture (pH 7.4), 0.4 ml. N HCl, and 0.2 ml. of .25 per cent eserine sulphate. This solution has a pH between 2 and 3. After the tissue had been homogenized, it was further diluted with the above solution until each milliliter of final suspension contained approximately 50 mgm. of tissue. The mixture was then boiled for 2 minutes and placed in a refrigerator to cool. When the preparation was ready to be assayed, it was centrifuged for 5 minutes at 2,000 R.P.M., and the clear liquid decanted off. This solution was made slightly alkaline



(7.2-7.4) with N NaOH and the acetylcholine content estimated on an eserized rectus abdominis muscle of the frog. Calculations were made in terms of the gamma of acetylcholine equivalent of acetylcholine chloride per gram of fresh tissue.

**RESULTS AND DISCUSSION.** The cholinesterase activity was measured in the retina from the 8th day of incubation until 2 days after hatching. The results for three series of experiments are presented graphically in figure 1, curve A. Each point in the curve represents the average values for a number of experiments at each stage of incubation. It will be noted that the cholinesterase activity

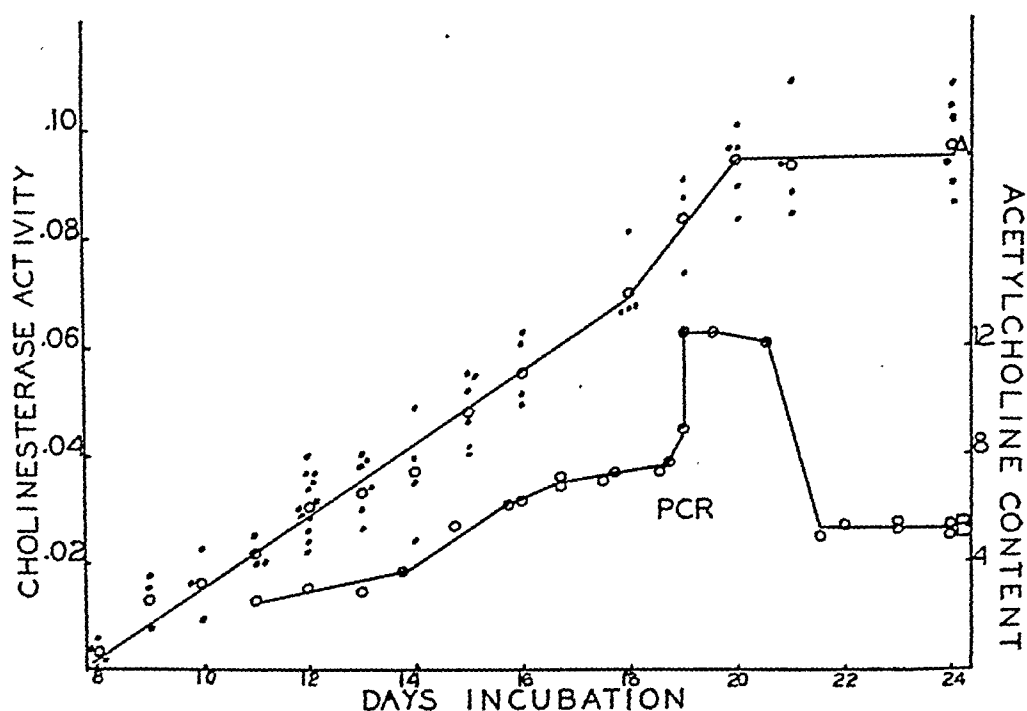


Fig. 1. Curve A. Cholinesterase activity expressed as acid equivalent (ml. 0.01 N HCl) produced by enzymatic hydrolysis per milligram fresh tissue per hour. Circles represent the mean and solid dots the individual analysis for a given incubation period.

Curve B. Acetylcholine content expressed as gamma equivalent of acetylcholine chloride per gram of fresh tissue. Points on curve individual experiments.

PCR—Pupillary constrictor reflex.

increases at a relatively constant rate from the 8th day to the 18th day of incubation at which point it takes a rather sudden rise until the 20th day when it levels off.

Since the increase in cholinesterase activity between the 18th and 20th days appears to be significant, a similar study of acetylcholine content was made. Inasmuch as there was no assurance that acetylcholine content parallels cholinesterase activity, this series was extended over a similar period of incubation.

Figure 1, curve B, presents the accumulated data from 3 series of eggs spaced at two week intervals. Attention is directed to the somewhat gradual increase in acetylcholine content between the 11th to 19th days. On the 19th day, how-

ever, there is an enormous increase from 7.8 gamma to a high of 12.5 gamma per gram of tissue. This level was then maintained until after hatching which took place between 21 and 22 days. Measurements made as early as 5 hours after the chick emerged from the shell revealed a sudden decrease in the acetylcholine content to approximately 5 gamma per gram of tissue. This level was maintained for 2 days after hatching at which point the experiment was terminated.

Under normal conditions it might be assumed that the retina does not function prior to hatching since development takes place in relative darkness. This does not mean that the retina is not sufficiently developed to function sooner if it is stimulated. Since the pupillary constrictor reflex is closely associated with the stimulation of the retina, experiments were designed to determine at what stage of incubation this reflex could be elicited. Embryos were partially removed from the egg in stages beginning with the 12th day of incubation and placed under the dissecting microscope. The eyelids were quickly dissected away and an intense beam of light from a microscope lamp was flashed on and off at short intervals. When no reflex could be elicited through this method, the top of the skull was removed and the brain explored with stimulating electrodes attached to a Harvard inductorium delivering relatively weak shocks. Constrictions were obtained by means of electrical stimulation of the brain as early as 14 days of incubation. However, the first sign of reflex stimulation occurred between 18 and 19 days at which time 2 or 3 small twitches were observed. Definite constrictions were always obtained at the 19th day. On the basis of this experimental evidence, it must be assumed that the neural units in the retina have matured by 19 days and can function as conductors of impulses.

Since an attempt is being made in this study to correlate functional activity with cholinesterase activity and acetylcholine content, attention is again directed to figure 1. It will be noted that the stage at which the pupillary constrictor reflex (*PCR*) can first be elicited by stimulation of the retina corresponds almost exactly with the sharp rise in acetylcholine and the smaller increase in cholinesterase activity. Thus, it would appear that an increase in both acetylcholine and cholinesterase is associated in some way with the beginning of the functional activity of the neural units in the retina.

A rather interesting finding which has developed from this study is the enormous drop in acetylcholine in the retina immediately after hatching. A similar decrease was reported by Szepsemwol and Caretti (1942) in later stages of development in chick brains. They found it to be especially marked in the medulla, cerebellum and cerebrum.

The data on the chick retina definitely reveal two significant changes in the acetylcholine content during the later stages of differentiation. The first, a marked increase at about 19 days of incubation and secondly, a very sharp decrease at hatching time when the eye is assumed to be functioning. These findings could indicate that the initial establishment of synaptic function may depend upon a relatively high intensity of stimulation. In other words, newly differentiated synapses may require higher threshold stimuli than do established functional synapses. This higher intensity of stimulation might be obtained by

the release of a larger quantity of acetylcholine from some reserve source. Such a reserve could well be in the form of a relatively high concentration of "bound" acetylcholine in the terminal boutons of the synapse. Then the marked fall in acetylcholine content at hatching time could be due to the depletion of the "bound reserve" which had been built up primarily for the purpose of establishing conduction through the synapses.

#### SUMMARY AND CONCLUSION

1. A quantitative study has been made of the cholinesterase and acetylcholine content of the developing chick retina for the period of incubation covering the differentiation of the neural units.

2. From the 8th to the 18th day, cholinesterase activity gradually increases with incubation time. Between the 18th and 20th day a significant increase occurs after which no appreciable change takes place for the remaining period studied.

3. The acetylcholine content shows a more gradual increase from the 11th to the 19th day. Around the 19th day a very sharp increase takes place, reaching a maximum level of between 12 or 12.5 gamma per gram of tissue. This level is maintained until shortly after hatching when it falls to a new level of between 5 and 6 gamma per gram of retina.

4. The pupillary constrictor reflex was first elicited between the 18th and 19th day of incubation. This was taken as evidence that the neural units of the retina are sufficiently developed at that time to function as conductors of impulses.

5. There appears to be a close relationship between the increase in cholinesterase and acetylcholine content and the maturation of the neural units in the developing chick retina.

6. It has been suggested that the sharp increase in acetylcholine content may be a "reserve" built up to facilitate the establishment of functional synapses.

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# A COMPARISON OF THE NUTRITIVE VALUE OF FATS WHEN FED ALONE OR WHEN FED WITH SUCROSE OR LACTOSE<sup>1</sup>

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That rats may subsist for prolonged periods of time on a diet consisting of a single foodstuff has been demonstrated by a number of investigators (1-3). Furthermore, physiological interrelationships may frequently be demonstrated far more efficiently under these simplified conditions than when animals are fed a more complete diet. Thus Richter and Rice (4) have demonstrated the specific rôle of thiamine hydrochloride in the utilization of carbohydrate by the rat, and Ershoff (5) has confirmed with the single food choice method the cataractogenic effects of galactose. The method may be employed as a tool useful in indicating possible interrelationships subject to confirmation by other experimental procedures. In view of the differences reported in the literature for the nutritive value of fats when lactose was the carbohydrate employed along with a complete diet (Boutwell et al., 6-8; Deuel et al., 9-11), it seemed desirable to reinvestigate the problem under the simplified conditions of the single food choice technique. Accordingly the effects of specific fats were determined on the length of survival of rats when fed as the sole source of calories or in combination with lactose or sucrose.

**PROCEDURE AND RESULTS.** *Experiments on carbohydrate-fat interrelationships.* Male and female rats of the Sprague-Dawley strain were raised to maturity on a stock ration and were selected for the following experiment at approximately 75 days of age. Animals were kept in individual metal cages with raised screen bottoms to prevent access to feces, and sufficient food was administered to assure *ad lib* feeding. The diets consisted of lactose or sucrose alone or mixtures of these sugars and test fats in the proportion of 70 per cent:30 per cent. The fats employed consisted of cottonseed oil, corn oil, margarine fat and butter fat.<sup>2</sup> The diets were prepared weekly and kept under refrigeration. The animals were fed fresh food daily. Six rats of each sex were used in each group. Room temperature for the present and subsequent experiments ranged from 66 to 75 degrees F. and humidity from 43 to 61 per cent. Results are summarized in table 1.

*Experiments with fats alone.* In the following experiment rats were fed diets consisting of a single fat.<sup>2</sup> Two experimental groups were employed consisting

<sup>1</sup> The subject matter of this paper has been undertaken in co-operation with the Quartermaster Corps Committee on Food Research.

<sup>2</sup> The fats were from the following sources: Cottonseed oil (Wesson oil), Wesson Oil and Snowdrift Sales Co., New Orleans, La.; Wheat germ oil, Vio Bin Corp., Monticello, Ill.; Soy bean oil, Tama Trading Co., Los Angeles, Calif.; Margarine fat, Best Foods, Inc.,

of female rats of the University of Southern California strain (series I) and male and female rats of the Sprague-Dawley strain (series II). Animals were raised to maturity on a stock ration and were selected for the present experiment at approximately 120 days of age for series I and 75 days of age for series II. All diets were fed *ad lib.* Six rats were used in each group. The data on length of

TABLE 1

*Summary table giving length of survival of rats fed diets consisting of lactose or sucrose alone or a mixture containing 70 per cent of lactose or sucrose with 30 per cent of different fats*

FAT OR OIL FED WITH CARBOHYDRATE	SEX	BODY WEIGHT		LENGTH OF SURVIVAL	
		Start	End	Individual tests	Average*
Lactose tests					
		grams	grams	days	days
Lactose only.....	M	183.8	118.5	8, 8, 9, 10, 12, 14	10.2 ±0.9
Butter.....	M	187.0	92.2	17, 24, 25, 28, 35, 41	28.3 ±3.2
Margarine.....	M	183.2	95.2	15, 31, 32, 34, 35, 35	30.3 ±2.9
Corn.....	M	181.0	114.8	15, 15, 17, 18, 18, 20	17.2 ±0.7
Cottonseed.....	M	177.4	109.6	9, 9, 10, 17, 17, 22	14.0 ±0.5
Lactose only.....	F	151.0	102.5	6, 6, 7, 8, 8, 9	7.3 ±0.5
Butter.....	F	149.3	86.6	13, 20, 23, 23, 24, 24	21.2 ±1.6
Margarine.....	F	149.2	80.0	9, 19, 27, 29, 30, 35	24.8 ±3.5
Corn.....	F	143.3	99.7	12, 13, 13, 16, 16, 18	14.7 ±0.9
Cottonseed.....	F	150.2	102.8	9, 11, 11, 15, 16, 17	13.2 ±1.2
Sucrose tests					
Sucrose only.....	M	186.8	88.3	33, 34, 36, 36, 38, 40	36.2 ±0.9
Butter.....	M	191.7	79.3	43, 44, 46, 48, 51	46.3 ±1.1
Margarine.....	M	187.3	89.3	30, 38, 41, 42, 47, 55	42.2 ±3.1
Corn.....	M	199.0	83.7	44, 44, 46, 47, 50, 50	46.8 ±1.0
Cottonseed.....	M	184.3	94.4	33, 36, 42, 44, 45, 49	41.5 ±2.2
Sucrose only.....	F	143.8	73.0	32, 33, 35, 36, 38, 39	35.5 ±1.0
Butter.....	F	137.8	64.3	35, 35, 37, 39, 42, 43	38.5 ±1.3
Margarine.....	F	138.3	64.5	30, 33, 34, 34, 36, 39	34.3 ±1.1
Corn.....	F	134.5	67.0	32, 33, 37, 39, 46, 47	39.0 ±2.4
Cottonseed.....	F	136.2	66.2	26, 29, 32, 36, 36, 39	33.0 ±1.8

\* Including standard error of the mean calculated as follows:  $\sqrt{\frac{ed^2}{n}}/\sqrt{n}$  where  $d$  is the deviation from the mean and  $n$  is the number of observations.

survival are summarized in table 2 while the results on food intake of series I are given in table 3.

New York, N. Y.; Corn oil, Corn Products Refining Co., Argo, Ill.; Olive oil, Parodi, Erminio and Co., San Francisco, Calif.; Lard, Luer Packing Co., Los Angeles, Calif.; Butter fat, Challenge Creamery Co., Los Angeles, Calif. Butter fat and margarine fat were prepared as described earlier (9).

TABLE 2

*The length of survival of rats fed diets ad libitum consisting of a single fat*

FAT OR OIL FED	BODY WEIGHT		LENGTH OF SURVIVAL	
	Start	End	Individual tests	Average*
Series 1 (Female rats)				
	grams	grams	days	days
Cottonseed.....	189.7	93.8	15, 17, 18, 24, 30, 37	23.5 ±3.2
Wheat germ.....	190.0	87.0	10, 18, 23, 26, 43, 46	27.7 ±5.3
Soybean.....	191.8	86.6	22, 23, 23, 29, 33, 37	27.8 ±2.3
Margarine.....	187.3	93.0	19, 22, 26, 30, 34, 44	29.2 ±3.4
Corn.....	188.7	81.2	27, 28, 31, 32, 32, 32	30.3 ±0.8
Olive†.....	197.0	94.0	15, 33, 33, 35, 38	30.8 ±3.6
Lard.....	188.3	97.3	18, 21, 33, 36, 42, 44	32.3 ±4.0
Butter.....	189.2	85.0	23, 33, 41, 42, 43, 44	37.7 ±3.1
Series 2 (Female rats)				
Cottonseed.....	155.2	94.5	11, 11, 13, 16, 16, 20	14.5 ±1.3
Corn.....	148.3	88.2	13, 13, 13, 18, 24, 24	17.5 ±2.0
Margarine.....	145.8	83.0	14, 17, 17, 18, 29, 29	20.7 ±2.5
Butter.....	144.7	72.5	32, 32, 32, 33, 41, 54	37.3 ±3.3
Series 2 (Male rats)				
Cottonseed.....	207.0	91.3	19, 29, 33, 37, 45, 47	35.0 ±4.0
Corn.....	198.6	94.0	29, 33, 34, 36, 44, 47	37.2 ±2.6
Margarine.....	197.0	96.2	23, 25, 25, 35, 42, 42	32.0 ±3.3
Butter.....	200.8	90.3	28, 30, 31, 41, 44, 47	36.8 ±3.1

\* Including the standard error of the mean calculated as follows:  $\sqrt{\frac{\epsilon d^2}{n}} / \sqrt{n}$  where  $d$  is the deviation from the mean and  $n$  is the number of observations.

† Only 5 rats in this test. One rat was discarded in this group inasmuch as its death on the third day of feeding did not appear to be due to experimental conditions.

TABLE 3

*The average fat consumption of series I rats fed the fat ad libitum*

FAT OR OIL FED	AVERAGE FOOD CONSUMPTION PER RAT*				
	1st week	2nd week	3rd week	4th week	Total for 4 weeks
	grams	grams	grams	grams	grams
Cottonseed.....	2.0 (6)	1.7 (5)	1.8 (3)	1.9 (2)	51.8
Wheat germ.....	2.4 (5)	1.7 (5)	2.1 (4)	1.7 (2)	55.3
Soybean.....	2.1 (6)	1.8 (6)	2.0 (6)	1.4 (3)	51.1
Margarine.....	2.3 (6)	2.9 (6)	2.9 (5)	2.4 (3)	73.5
Corn.....	1.0 (6)	1.8 (6)	1.8 (6)	1.8 (4)	44.8
Olive.....	2.4 (5)	2.3 (5)	1.8 (4)	1.6 (4)	56.7
Lard.....	3.5 (6)	3.7 (6)	3.3 (4)	2.0 (4)	87.5
Butter.....	3.0 (6)	3.3 (6)	3.5 (6)	2.3 (5)	84.7

\* Figures in parentheses represent the number of rats on which the average is based.

**DISCUSSION.** This is the first report to our knowledge of an investigation of carbohydrate-fat interrelationships with the single food choice method. Results indicate that when lactose was the dietary carbohydrate survival was significantly longer in both sexes on diets containing margarine or butter fat than on rations containing corn oil or cottonseed oil. With sucrose as the dietary carbohydrate no significant differences in length of survival were observed regardless of dietary fat. These experiments indicate again the poor nutritive value of lactose as a dietary carbohydrate, since survival on diets containing lactose either alone or in combination with fat was significantly less in all cases than that on rations containing sucrose, an observation in agreement with previous findings (2, 3, 5, 12).

When fat was fed as the sole component of the diet, no significant differences in length of survival were observed in male rats irrespective of whether butter fat, margarine fat, corn oil or cottonseed oil was fed. With female rats, however, best results were obtained on the butter fat diet. In the U.S.C. strain (series I) significant differences were found only between the butter fat and cottonseed oil groups. In series II, where the Sprague-Dawley strain was employed, animals receiving butter fat lived significantly longer than those on margarine fat, corn oil or cottonseed oil. These results are in agreement with the earlier report of Richter (2) although the sex and strain of his rats were not noted. This author reported the following average survival time in days with the various fats: butter, 53; olive oil, 47; lard, 28; wheat germ oil, 24; cod liver oil, 20; Crisco, 18; peanut oil, 17; perilla oil, 11.

The longer survival times in the present experiment as well as in the Richter tests were associated with a larger food intake. This relationship was also observed by Holt and Kadji (3). In the present experiment total food consumption was greatest in the lard and butter fat groups where the longest survival also was noted. With the six other fats investigated in which the survival time was somewhat shorter, the food intake was in each case markedly lower than that of the butter group. On an average the intake was only 65 per cent of that ingested by the butter-fed rats. However, in spite of the fact that survival was longest for the two groups whose food consumption was greatest, no correlation could be established in the vegetable fat series between the length of survival and the amount of food ingested.

One possible cause for the discrepancy in fat intake may have been the physical state of the fats. In the experiments where lactose-fat mixtures were fed, the two fats solid at room temperature were the ones giving the best results. In the tests with fat alone, the better results with butter, lard and in some cases margarine over the liquid fats may have been due in part to their consistency. Although only the butter and margarine contained vitamin A, due to the relatively short periods of the tests it is not considered that differences in the fat-soluble vitamin content were responsible for the variations in survival period. However, it is possible that requirements for some of the fat-soluble vitamins were augmented in the unusual dietary condition where fat alone was fed. Further

experiments are needed where an adequate intake of all the fat-soluble vitamins is assured.

An interesting finding in the present tests was the marked variation in the survival time of male and female rats on diets containing various fats as the sole source of calories. Although no significant differences in length of survival were noted between male and female rats on butter fat alone, survival of male rats on margarine fat, corn oil or cottonseed oil averaged in some cases as much as twice that of female rats on the same fats. Although the cause for this difference is not immediately apparent, differences in carbohydrate and fat metabolism between the sexes are known. Thus the male stores more glycogen or retains it longer in fasting (Deuel et al., 13) while the female is more susceptible to ketonuria (Butts and Deuel, 14).

#### SUMMARY

1. Male and female rats were fed diets containing 70 per cent sugar (lactose or sucrose) and 30 per cent fat (butter fat, margarine fat, corn oil or cottonseed oil). When lactose was the dietary carbohydrate, survival was significantly longer on rations containing margarine fat or butter fat than on diets containing corn oil or cottonseed oil. With sucrose as the dietary carbohydrate, no significant differences in length of survival were observed.

2. Male and female rats were fed diets consisting of a single fat. With female rats of the U.S.C. strain, length of survival was longest with butter fat as compared with lard, olive oil, corn oil, margarine fat, soybean oil, wheat germ oil and cottonseed oil but the only significant difference was between the butter fat and cottonseed oil groups. With females of the Sprague-Dawley strain, results on butter were significantly better than with margarine fat, corn oil or cottonseed oil. No significant differences in length of survival were observed in male rats fed the above fats.

3. Periods of survival of male rats on sugar-fat mixtures as well as on exclusive fat diets were longer than those of female rats. In a number of cases these differences were significant.

4. There is some evidence of a strain difference in survival periods. Female rats of the U.S.C. strain lived longer on the vegetable fats than rats of the Sprague-Dawley strain.

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# THIAMINE DEFICIENCY AND THE SPECIFIC DYNAMIC EFFECT OF A DIET HIGH IN CARBOHYDRATE

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The specific dynamic action of glucose is increased when this is fed with large amounts of thiamine (Ring, 1943), and it is of interest to inquire whether a deficiency of thiamine will reduce the S. D. A. of a diet high in carbohydrate.

**METHOD.** Small rats weighing about 40 grams were used for this work because these develop nutritional deficiencies more quickly than older animals. The diet used is shown below.

Vitamin-free casein.....	214 grams	Cod liver oil.....	20 grams
Corn oil.....	40 grams	Succinylsulfathiazole.....	10 grams
Sucrose.....	626 grams	Choline hydrochloride <sup>1</sup> .....	1 gram
Autoclaved yeast.....	60 grams	Inositol <sup>1</sup> .....	1 gram
Salt mixture (No. I, U.S.P. XII, p. 637).....	40 grams	Calcium pantothenate <sup>1</sup> .....	20 mgm.

One group of rats received no thiamine with the above mixture; a second group received 1 mgm. of thiamine<sup>1</sup> per kgm. of food; a third group, 2 mgm., and a fourth group, 10 mgm. This food was fed throughout the experimental period, and the S. D. A. was measured after feeding one of these mixtures. Using the Haldane principle, the metabolism was determined for seven hours while the animals were fasting, and the next day for eight hours after the ingestion of food. The S. D. A. was calculated by subtracting the figures for the metabolism of fasting animals from those determined after a measured amount of food was eaten. It was assumed that the protein was burned in the proportion in which it was ingested. The possible error from this assumption is believed to be unimportant.

**RESULTS.** The average gain in weight during the first three weeks, while subsisting on one of the above diets, was as follows:

Rats receiving no thiamine.....	36.1 per cent increase
Rats receiving 1 mgm. of thiamine per kgm. of food.....	60.3 per cent increase
Rats receiving 2 mgm. of thiamine per kgm. of food.....	60.0 per cent increase
Rats receiving 10 mgm. of thiamine per kgm. of food.....	63.6 per cent increase

The rats ingesting the food lacking thiamine survived for about four weeks. If these animals fasted after they had subsisted three and one-half weeks on this diet, death occurred within one day.

At the end of one week on the diet lacking in thiamine, the S. D. A. was not reduced. Measurements at that time showed the S. D. A. to be 7.32 per cent and 6.63 per cent for such rats. This is within the range of variation for the

<sup>1</sup> Kindly provided by Merck and Company, Rahway, N. J.

S. D. A. of a diet containing adequate amounts of thiamine. Table 1 shows the results after all of the rats had been living on one of the above rations for two weeks or more. Since the highest figure for S. D. A. observed when no thiamine was supplied (4.90 per cent) was lower than the lowest when adequate thiamine was provided (6.47 per cent) the S. D. A. must be lower than normal on a diet lacking in thiamine. From our results, it also appears that the diet containing 1 mgm. of thiamine per kgm. does not show as large an S. D. A. as the control with 10 mgm. of thiamine but there is probably no difference between observations for rats receiving 2 mgm. of thiamine per kgm. of food and those receiving 10 mgm.

In measuring the S. D. A., no attempt was made to keep the food intake constant and it will be noted that the rats with inadequate thiamine intake ate less food than the others. That this might account for the lower S. D. A. is sug-

TABLE 1  
*The effect of the thiamine level in the diet on the specific dynamic action*

DATE	NO THIAMINE		1 MGm. THIAMINE*		2 MGm. THIAMINE*		10 MGm. THIAMINE*	
	Avg. food intake**	S. D. A.	Avg. food intake**	S. D. A.	Avg. food intake**	S. D. A.	Avg. food intake**	S. D. A.
		per cent		per cent		per cent		per cent
Apr. 2-3.....	0.442	4.90	0.671	6.71	0.880	7.69	0.734	8.41
Apr. 9-10.....	0.458	3.48	0.441	5.25	0.676	5.81	0.788	6.47
Apr. 16-17.....			0.474	4.73	0.701	6.57	0.870	6.52
Apr. 30-May 1.	0.600	2.36	0.676	5.11	0.293	4.50	0.577	8.92
May 7-8.....	0.386	3.03	0.458	5.98			0.540	6.27
May 14-15.....			0.547	6.60	0.488	8.97	0.610	8.44
Average.....	0.471	3.44±0.37	0.544	5.73±0.23	0.607	6.70±0.50	0.687	7.50±0.31

\* Per kgm. of food.

\*\* Per 100 grams of rat per hour.

gested by the work of Forbes, Kriss and Miller (1934), who showed that the S. D. A. was greater with a large intake of food than with a small one. However, one can see that our observations show no correlation between the amount of food ingested and the S. D. A. observed. The differences in food intake were apparently not large enough to produce measurable changes in S. D. A.

The respiratory quotients after high carbohydrate ingestion were, of course, elevated. The rats receiving no thiamine showed an average R. Q. of 0.88; with 1 mgm. of thiamine per kilo of food, 0.92; with 2 mgm., 0.88; with 10 mgm., 0.94. There is no significant difference in the respiratory quotients of normally fed rats and those on thiamine deficient diets. It is probable that the large difference in respiratory quotients reported by Church and Whipple (1937) occurs only during the terminal period of severe deficiency.

The metabolic measurements on these rats while fasting gave approximately the same figures regardless of previous diet. Those receiving no thiamine ex-

pended 0.744 Cal. per 100 grams per hour; those getting 1 mgm., 0.742 Cal.; 2 mgm., 0.827 Cal.; and 10 mgm., 0.732 Cal. This confirms the earlier observations of Drummond and Marrian (1926) showing that the resting metabolism remains normal until the final phase when marked malnutrition occurs.

Since thiamine is important in carbohydrate metabolism, it is probable that it affects the S. D. A. of this foodstuff primarily. Baur (1929) and others subsequently have produced evidence that the S. D. A. of carbohydrate is due to its conversion to and storage as glycogen. In thiamine deficiency, according to Harper (1942), there is a decrease in glycogenesis.

#### CONCLUSIONS

1. The S. D. A. of a diet high in carbohydrate and containing adequate thiamine is about twice as large as that of a similar diet without thiamine (see table 1).

2. One milligram of thiamine added to a kilogram of this diet did not produce as large an S. D. A. as 2 or more milligrams (see table 1).

3. The thiamine deficient rats at the time studied showed no measurable decrease in ability to use carbohydrate as judged from respiratory quotients.

4. The total metabolism of fasting was as high as that in the control rats.

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# RENAL REGULATION OF WATER AND SOME ELECTROLYTES IN MAN, WITH SPECIAL REFERENCE TO THEIR RELATIVE RETENTION AND EXCRETION<sup>1</sup>

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The present report concerns renal responses of man to steady intakes of water, with and without dissolved electrolytes.

**PROCEDURES.** *a. Intravenous experiments.* In a group of 12 experiments, 8 male subjects at rest were infused with salt solutions ranging from 0 to 344 m.eq. of NaCl per liter. Solutions of 75 m.eq./l. or less were fortified osmotically with 4 per cent glucose. No glycosuria was observed. The infusion was regulated by a pump which maintained an input rate of approximately 7 cc./min. (known accurately for each experiment) for 7 hours, providing approximately a 2940 cc. intake. The bladder was emptied at a known time approximately one hour before the subject reported for the experiment, and again at the beginning of the infusion, providing a control sample. The bladder was emptied every hour thereafter and aliquots were analyzed for pH, Na, K,  $\text{NH}_3$ , Cl, and  $\text{HCO}_3$ . Conditions were not conducive to sweating.

*b. Oral experiments.* In a group of 22 experiments, 13 male subjects at rest drank water or solutions of KCl,  $\text{KHCO}_3$ ,  $\text{NH}_4\text{Cl}$ , or  $\text{NaHCO}_3$  of specified concentration, taking 70 cc. every ten minutes (expressed as 7 cc./min.) for 7 hours. Other conditions are as in *a*.

Two blood samples were drawn in each experiment, one control and one in the 7th hour. In all experiments the subjects ranged from 19 to 31 years of age and from 61 to 93 kgm. in weight.

**METHODS.** All Na and K determinations were made with a Perkin-Elmer flame photometer. When Na or K and the associated anion of the salt in the intake fluids were determined, the average per cent deviation from the mean was 3.0 per cent for Na and 2.2 per cent for K. No deviation was over 9 per cent. It was necessary to introduce a finely controlled gas regulating valve in series with the photometer's air supply in order to maintain the constancy of pressure required for reproducible analyses.

Total  $\text{CO}_2$  was measured with a Van Slyke manometric apparatus, and  $\text{HCO}_3$  was estimated by the Henderson-Hasselbalch equation. Urinary pH was determined with a Beckman pH meter.  $\text{NH}_3$  was determined by Folin's permutit method. At the start of absorption of  $\text{NH}_3$  the liquid in contact with permutit was just acidified to methyl red. A modified Volhard method was used for chloride.

Plasma from blood samples collected without special precautions other than

<sup>1</sup> Aided by a grant from the Dazian Foundation for Medical Research.

covering during centrifugation, and anticoagulated with ammonium oxalate, was analyzed for Na, K, Cl and  $\text{HCO}_3$  (assumed pH of 7.35).

Urines were analyzed for  $\text{NH}_3$  within one hour of collection; for pH, when they had come to room temperature. Collection of urine was carried out in 500 cc. graduates, with minimal turbulence, and a sample transferred to a large test tube which was filled and stoppered. With alkaline urines, the loss of total  $\text{CO}_2$  was not appreciable as judged by comparing with some samples led under oil through a Gooch rubber tube held hermetically to the penis, and drawn directly into pipettes for analysis. No significant differences in total  $\text{CO}_2$  were found. Also, analyses of urine before and after turbulent handling showed no appreciable loss of total  $\text{CO}_2$  induced. However, the pH of such urines rose a fraction of a pH unit (0.68 with prolonged agitation) after turbulent handling

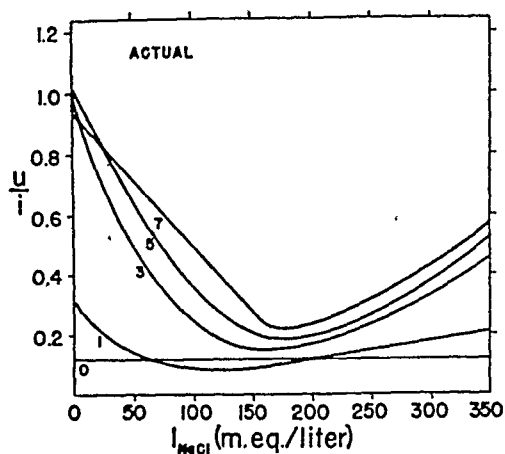


Fig. 1

Fig. 1. Ratio of rate of urinary flow,  $u$ , to fluid intake rate,  $i$ , plotted against concentration of NaCl ( $I_{\text{NaCl}}$ ) in infusion fluid during control period (0 time) and during 1st, 3rd, 5th and 7th hours (time printed beside curves).

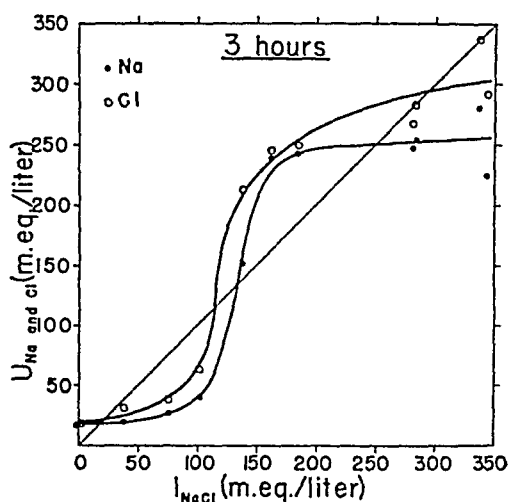


Fig. 2

Fig. 2. Urinary Na and Cl concentration ( $U$ ) at the 3rd hour plotted against concentration of NaCl ( $I_{\text{NaCl}}$ ) in intake fluid.

and thus the pH values and the calculated  $\text{HCO}_3$  values reported for urines collected without turbulence may be somewhat high.

**RESULTS.** a. *With NaCl.* Figure 1 shows the ratio of urinary output rate to intake rate ( $u/i$ ) in relation to the infusion concentration ( $I$ ). The family of curves shows the progression of the steady state toward equilibrium of intake and output. The 7th hour data for these variables which are included in table 1 form an intermediate step between the 3 and 5 hour data presented here (fig. 1) and the data of Stewart and Rourke (1942) in which equilibrium was attained after several days of continuous infusion with 0.9 per cent NaCl. Equilibrium probably can only be reached with infusion concentrations between the minimal isorrheic concentration (MIC) and limiting isorrheic concentration (LIC) (Wolf, 1945a). The minimal  $u/i$  values obtained from infused solutions whose

concentration of NaCl lies in the range 100-200 m.eq./l. characterized the solutions most hydropigenous per unit volume infused.

TABLE 1

Intravenous NaCl. Infusion rate,  $i$  = ca. 7 cc./min. Urine flow,  $u$ , is in cc./min. Concentration of infusion fluid,  $I$ , and of urine,  $U$ , is in m.eq./l. Excretion rate,  $uU$ , is in micro-eq./min. Net load of water,  $L_{H_2O}$ , at end of 7th hour is in cc. and was obtained by subtracting 294 cc. from the total water intake minus total urine output at that time.

SUBJECT	$I_{NaCl}$	TIME	$u$	$\frac{u}{i}$	$uU_{Na}$	$uU_{Cl}$	$uU_{HCO_3}$	$uU_{NH_3}$	$uU_K$	pH	$L_{H_2O}$
		min.									
J. H.	0.0	0	0.82	0.11	156	196	0.2	47.0	58.5	5.28	333
		420	5.81	0.80	58.1	75.6	4.1	61.0	40.7	6.00	
G. N.	0.0	0									-80
		420	6.84	0.96	123	75.2	4.0	46.5	58.7	5.92	
L. S.	37.5	0	1.13	0.17	187	243	8.0	39.1	79.7	6.60	36
		420	5.10	0.75	133	158	17.4	42.8	51.0	6.58	
L. G.	75.0	0	0.97	0.14	260	284	0.3	43.6	51.2	5.61	1218
		420	3.88	0.55	140	147	0.4	47.4	24.1	5.52	
R. M.	102	0	0.57	0.08	54.6	100	0.2	21.2	80.5	5.44	1692
		420	3.90	0.56	187	156	1.6	35.4	84.4	5.68	
R. M.	138	0	0.72	0.11	86.5	173	0.1	29.5	96.5	5.26	2059
		420	2.76	0.40	199	224	0.8	34.0	106	5.58	
R. M.	162	0	0.71	0.10	131	176	0.1	37.8	78.0	5.10	2254
		420	1.28	0.19	225	249	0.1	44.4	80.3	5.03	
R. M.	185	0	0.96	0.13	92	174	0.1	28.0	88.0	4.86	2164
		420	1.58	0.22	316	410	0.3	31.4	109	5.10	
A. B.	283	0	0.45	0.06	68.4	77.9	0.1	45.0	35.3	5.36	1264
		420	4.02	0.55	1160	1280	6.4	62.4	132	6.06	
A. W.	284	0	0.48	0.07	42.2	112	0.01	35.6	33.8		1895
		420	2.78	0.39	834	836	0.02	58.4	121		
A. B.	338	0	1.89	0.26	469	284	8.3	47.2	130	6.40	1638
		420	3.87	0.54	1110	1330	10.8	36.8	186	6.20	
D. S.	344	0	0.46	0.06	40.5	85	0.1	55.9	32.4	5.06	2044
		420	2.50	0.35	760	865	0.5	45.7	111	5.37	

Figures 2 and 3 relate the urinary concentration ( $U$ ) of Na and of Cl at the 3rd and 7th hours respectively, to the concentration of the infused fluid ( $I$ ). The characteristic sigmoid curve found by Wolf (1943) for Cl holds also for Na.

An MIC of approximately 15 m.eq./l. is found for both Na and Cl at the 7th hour.

The equation of steady state, uncorrected for insensible water loss, is

$$\frac{u}{i} = \frac{(A_T)_{Na} - I_{Na}}{(A_T)_{Na} - U_{Na}} = \frac{(A_T)_{Cl} - I_{Cl}}{(A_T)_{Cl} - U_{Cl}} \quad (1)$$

where  $u$  and  $i$  are respectively the rate of urine output and of fluid intake in cc./min.;  $U$  and  $I$  are respectively the urinary and infusion fluid concentrations of Na or of Cl; and  $A_T$  is the threshold of retention (normal plasma concentration) found to average 140 m.eq./l. for Na and 98 m.eq./l. for Cl in control plasma samples. Figure 4 gives theoretical values for  $u/i$  computed from

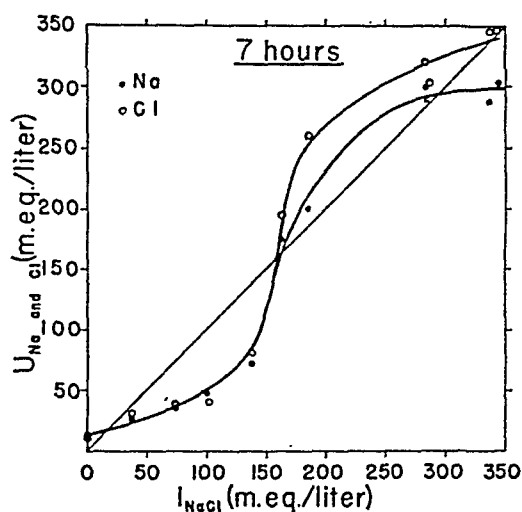


Fig. 3

Fig. 3. Urinary Na and Cl concentration ( $U$ ) at the 7th hour plotted against concentration of NaCl ( $I_{NaCl}$ ) in intake fluid.

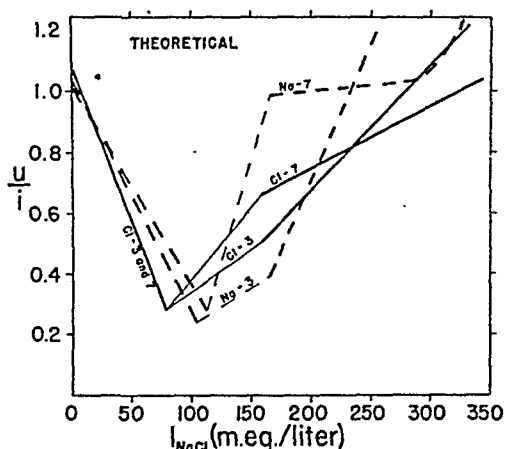


Fig. 4

Fig. 4. Theoretical ratio of rate of urinary flow,  $u$ , to fluid intake rate,  $i$ , plotted against concentration of NaCl ( $I_{NaCl}$ ) in infusion fluid. Computed from steady state equation, corrected for insensible water loss assumed to be 0.7 cc/min. Based on 3rd and 7th hour urinary concentrations of Na and Cl (time printed beside curves). Thresholds of retention (normal plasma concentration of control period) were found to be 140 m.eq./l. for Na and 98 m.eq./l. for Cl. Thin lines connecting heavy ones represent the region in which the steady state equation cannot be solved with accuracy.

equation (1) corrected for an assumed insensible loss of 0.7 cc./min. (Wolf, 1945; 1945a). Comparison with figure 1 affirms the discrepancy between theoretical and actual values, the significance of which will be discussed. By equation (1) when  $u/i$  is unity, the MIC for Na and for Cl should be the same; and the LIC for Na and for Cl should be the same, providing normal plasma concentrations were maintained constant, i.e.,  $I_{Cl} = I_{Na} = U_{Na} = U_{Cl}$ . Only the 7th hour MIC, not the LIC, is actually the same for Na and for Cl.

Peters and Van Slyke (1935) suggest that with infusions of physiological salt solutions whose chloride concentration is higher than plasma, chloride, which is thrown away in the urine in excess of Na, may be carried in combination with ammonium ion produced in the kidney's rôle as acid-base adjustor. No evi-



dence for this has been found in normal man. By averaging the excretion rates of  $\text{NH}_3$  after "hypotonic" and "hypertonic"  $\text{NaCl}$  infusions, no significant change in excretion rate of  $\text{NH}_3$  is found in the course of 7 hours (fig. 5b).

A marked increase in the excretion of  $\text{K}$  (fig. 5b) is obtained with hypertonic  $\text{NaCl}$  infusions as compared with hypotonic ones. This increase in  $\text{K}$  excretion with increase in  $\text{NaCl}$  infusion concentration is otherwise shown in figure 5a. It is slightly greater than the excess of  $\text{Cl}$  excreted over  $\text{Na}$ . Elkinton and Winkler (1944) suggest this phenomenon is not due to a specific effect of some

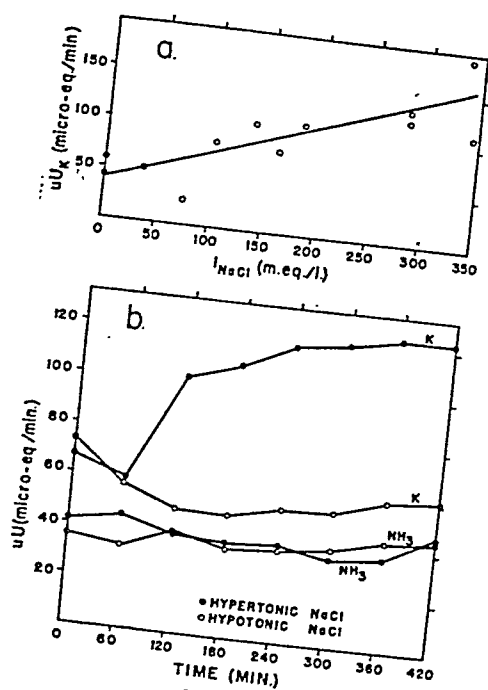


Fig. 5

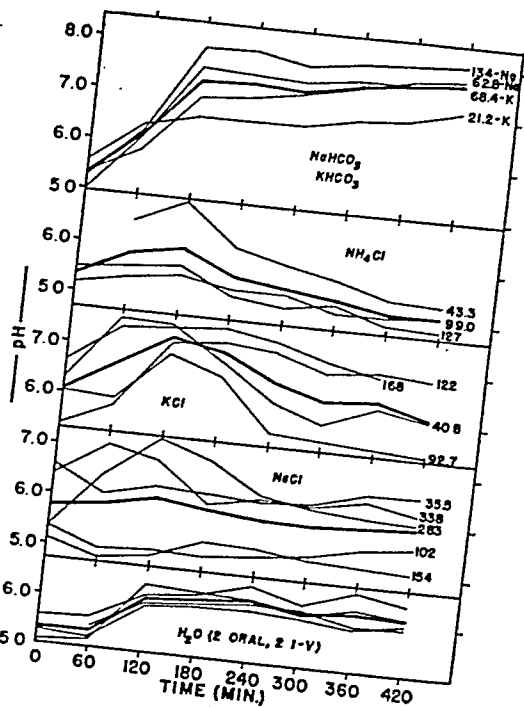


Fig. 6

Fig. 5a. Rate of excretion of  $\text{K}$  ( $uU_K$ ) during the 7th hour plotted against concentration of  $\text{NaCl}$  ( $I_{\text{NaCl}}$ ) in infusion fluid.

Fig. 5b. Rates of excretion of  $\text{K}$  and of  $\text{NH}_3$  plotted against the duration of  $\text{NaCl}$  infusion. Each point is the of average 6 cases, the hypotonic ones representing the 6 concentrations of  $\text{NaCl}$  from 0 to 138 m.eq./l., and the hypertonic ones, representing the 6 concentrations from 162 to 344 m.eq./l. (table 1).

Fig. 6. pH of urine as it varied with the duration of the experiment. Thin lines connect actual values of urinary pH resulting from solutions whose concentrations are printed at the right. Heavy lines connect averages of thin line data.

one salt. Hypertonicity of other injected salts or water deprivation may bring on an excess loss of  $\text{K}$  (Wiley and Wiley, 1933). Stewart and Rourke (1942) report higher excretion rates for  $\text{K}$  with 0.9 per cent  $\text{NaCl}$  infusions than with those of 5 per cent glucose-water.

Figure 6 shows the pH of urine is not significantly modified by the infusion of  $\text{NaCl}$  solutions which are not extremely dilute. Pure water intake brings on an alkaline tide (Carr, 1921; Brunton, 1933; Barnett and Blume, 1938).

b. With all salts. Table 2 includes data on solutions of  $\text{KCl}$ ,  $\text{KHCO}_3$ ,  $\text{NaHCO}_3$  and  $\text{NH}_4\text{Cl}$ . The effect of these solutions on urinary pH is shown in figure 6.

The alkalization of the urine induced by KCl solutions and previously described by Loeb et al. (1932), Keith et al. (1937), and others, is seen essentially as a transient alkaline tide whose magnitude depends in part on the concentration of KCl in the intake fluid. This tide is correlated with an increased  $\text{HCO}_3^-$  excretion, decreased  $\text{NH}_3$  excretion, and in these experiments, the rate of Cl excretion exceeded that of K (Wiley, Wiley and Waller, 1933). This contrasts with results reported for subjects on NaCl-poor diets by Loeb et al. (1932). The alkalization of the urine is unsatisfactorily explained by assuming simply that K is excreted more rapidly than Cl and therefore  $\text{HCO}_3^-$  makes up the electroneutrality. Whatever the basis for alkalization, the effect was transient in these experiments. Even  $\text{NH}_4\text{Cl}$  solutions produced brief alkaline tides, perhaps reflecting the effect of water diuresis.

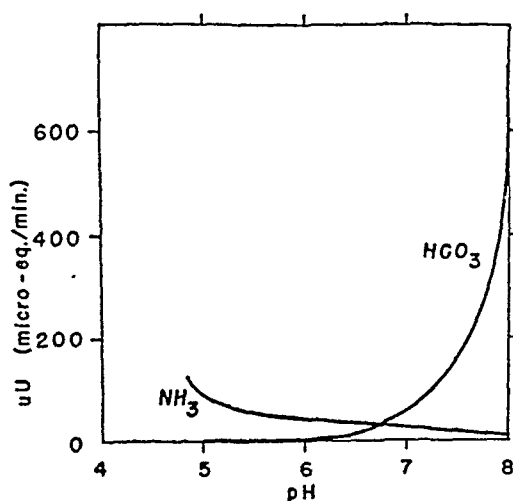


Fig. 7

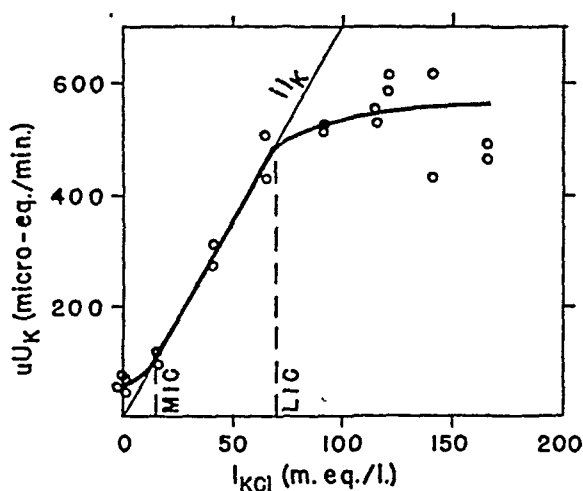


Fig. 9

Fig. 7. Rate of excretion of  $\text{NH}_3$  and of  $\text{HCO}_3^-$  at 7th hour, plotted against the pH of urine. Data taken from all experiments provide complete pH range.

Fig. 9. Rate of excretion of K ( $u_{\text{UK}}$ ) at the 6th and 7th hours plotted against intake fluid concentration of KCl ( $I_{\text{KCl}}$ ). The thin oblique line going through the origin is the locus of points ( $7 \times I_{\text{KCl}}$ ) representing the rate of intake of K ( $iI_{\text{KCl}}$ ). Between the MIC and the LIC of K, intake and output of K are equal.

Figure 7 shows the relations of  $\text{NH}_3$  and  $\text{HCO}_3^-$  excretion to urinary pH.  $\text{HCO}_3^-$  is found in appreciable quantities only between pH 6 and 8.  $\text{NH}_3$  is found throughout the entire pH range of urine (though on occasion its excretion was insignificantly different from zero). It was balanced equivalently by  $\text{HCO}_3^-$  at approximately pH 6.8. The gradual increase in  $\text{NH}_3$  excretion from alkaline to acid urines is not wholly consistent with the supposed rôle of  $\text{NH}_3$  excretion as a base saving mechanism. The first correlation of  $\text{NH}_3$  excretion appears to be with urinary pH.

Figure 8 indicates the load at the 7th hour (total intake minus total urinary output at the end of the 7th hr.) of each component studied. The effect of different concentrations of the different solutions used is specified. In the case of  $\text{NH}_3$ , the term "load" is used in an extended sense since the  $\text{NH}_3$  does not exist in the body as a load of  $\text{NH}_3$  or even of hydrogen ions, but as the rough equivalent

of a negative load of  $\text{HCO}_3$ . The  $\text{NaCl}$  administered was intravenous in order to permit an estimate of the systemic response to the higher intake concentrations which would have been deflected from absorption in the gut. Some imbalance of various components just prior to the experiment is reflected in the final loads on the graphs, but the following points are noteworthy.

1.  $\text{NaHCO}_3$  solutions induce less water retention than those of  $\text{NaCl}$  (oral  $\text{NaCl}$  solutions provide even less urine output than intravenous solutions when the concentration is over ca. 100 m.eq./l.).

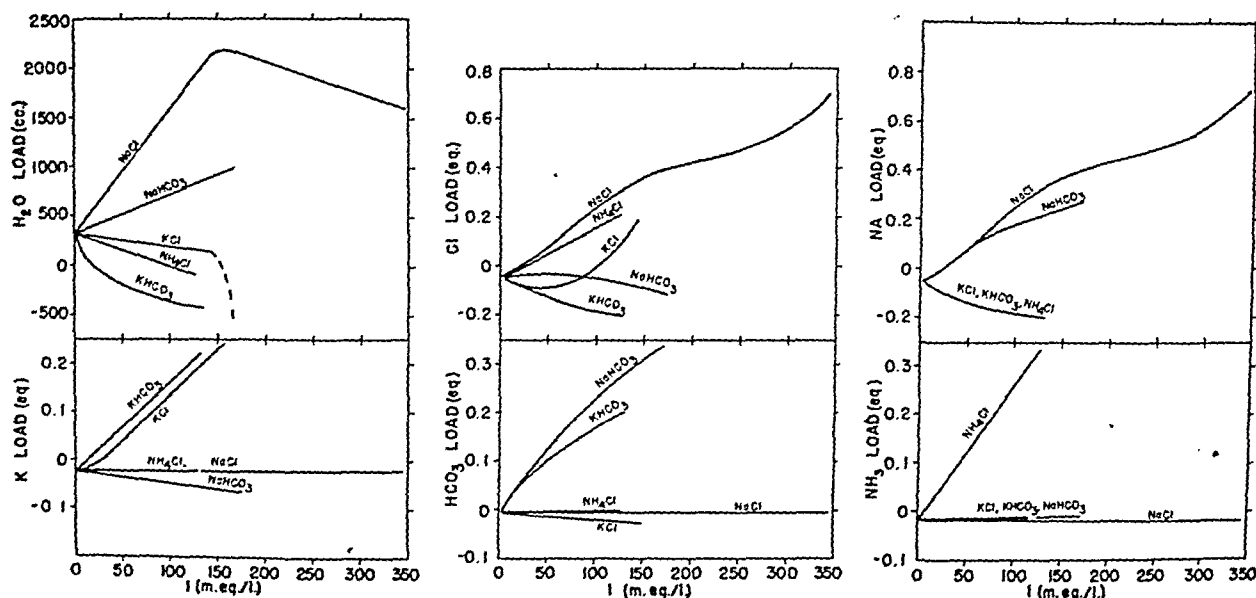


Fig. 8. Load of water (net, i.e., 294 cc. subtracted from difference between amount of fluid put in and amount of urine put out, by the end of the 7th hour), K, Cl,  $\text{HCO}_3$ , Na and  $\text{NH}_3$  at 7th hour, plotted against the concentration (I) of each substance administered (printed beside each curve). The broken line for KCl in the upper left hand graph indicates the severe ecuresis or absolute dehydration induced until vomiting stopped the experiment at the 349th minute. Good fit lines were drawn through actual points, not shown, but curves of small slope may or may not indicate significant trends. The "load" of  $\text{NH}_3$  was computed from intake and output without regard to its existence in the body.

2.  $\text{KHCO}_2$  solutions bring about the greatest depletion (negative loads) of water, Na, and Cl; they decrease the excretion of  $\text{NH}_3$  (less negative load of  $\text{NH}_3$  than with water).

3.  $\text{KHCO}_3$  replaced systemic  $\text{NaCl}$  almost quantitatively.

4.  $\text{KCl}$  solutions remove Na and  $\text{HCO}_3$ , and decrease the excretion of  $\text{NH}_3$ .

5.  $\text{NH}_3$  excretion is almost independent of  $\text{NaCl}$  concentration infused.

6.  $\text{NH}_4\text{Cl}$  solutions add Cl approximately to the extent that  $\text{KHCO}_3$  removes Cl; and their effects on plasma  $\text{HCO}_3$  are opposite (table 3).

Figure 9, relating excretion rate of K to the concentration of KCl ingested, shows a characteristically different curve than Na or Cl with  $\text{NaCl}$  solutions (figs. 2, 3). K reaches equilibrium of intake and output readily. It shows an MIC of ca. 15 m.eq./l., approximately the same magnitude as that of Na and

TABLE 2  
Oral intakes, 7 cc./min. Symbols as in table 1

SOLUTION AND SUBJECT	I	TIME	$\mu$	$\mu U_{Na}$	$\mu U_{Cl}$	$\mu U_{HCO_3}$	$\mu U_{NH_3}$	$\mu U_K$	pH	$L_{H_2O}$
		<i>min.</i>								
Water.....	0.0	0	0.29	27.8	60.4	0.03	24.0	31.6	5.09	
J. F.....		420	7.00	98.0	105	7.0	38.5	42.0	6.15	131
Water.....	0.0	0	0.87	102	183	0.35	34.7	83.1	5.56	
T. R.....		420	6.00	72.0	48.0	8.4	40.2	76.8	6.40	865
NaHCO <sub>3</sub> ....	36.1	0	0.53	115	143	0.9	20.9	63.5	6.11	
T. R.....		420	4.16	133	20.8	90.0	18.3	73.3	7.46	684
NaHCO <sub>3</sub> ....	62.8	0	0.53	55.1	62.5	0.01	49.0	47.5	5.31	
S. P.....		420	6.34	406	82.5	216	15.2	50.7	7.75	931
NaHCO <sub>3</sub> ....	92.7	0	0.73	130	156	24.2	12.9	130	7.30	
A. B.....		420	9.25	444	148	345	23.2	100	7.73	421
NaHCO <sub>3</sub> ....	135	0	0.49	49.0	97.5	0.01	36.8	77.0	5.23	
A. M.....		420	5.25	410	10.5	388	18.4	141	8.01	1165
NaHCO <sub>3</sub> ....	174	0	1.70	381	402	3.2	83.0	155	6.18	
A. B.....		420	6.56	931	204	711	12.5	114	8.01	465
KHCO <sub>3</sub> ....	21.2	0	0.58	130	168	0.2	35.2	50.1	5.58	
L. V.....		420	6.50	260	202	66.3	5.9	156	7.10	-168
KHCO <sub>3</sub> ....	51.4	0	0.78	93.5	160	0.1	35.8	71.1	4.80	
R. M.....		420	9.08	654	354	236	29.0	218	7.42	-135
KHCO <sub>3</sub> ....	68.4	0	0.88	91.6	117	0.01	30.8	67.0	4.96	
R. M.....		420	7.72	247	262	283	21.6	358	7.63	-400
KHCO <sub>3</sub> ....	130	0	1.03	264	226	96.2	6.7	95.6	7.85	
A. B.....		420	5.34	267	197	439	16.0	436	8.09	-298
NH <sub>4</sub> Cl.....	43.8	0	0.56	80.6			55.7	43.9		
A. B.....		420	9.36	450	402	1.9	48.8	37.5	5.31	-245
NH <sub>4</sub> Cl.....	99.0	0	0.74	231	196	1.1	48.9	33.2	5.18	
L. G.....		420	7.71	494	485	3.9	60.1	84.7	5.10	136
NH <sub>4</sub> Cl.....	127	0	0.37	53.4	81.4	0.1	25.2	51.0	5.49	
A. B.....		420	11.5	826	725	1.2	132	82.6	4.82	71
KCl.....	16.5	0								
L. D.....		420	7.25	116	218	8.0	59.5	94.1	6.21	-157
KCl.....	40.8	0	0.47	79.0	127	1.7	21.6	101	6.30	
L. V.....		420	7.50	240	397	6.0	37.5	276	6.03	-63

TABLE 2—Concluded

SOLUTION AND SUBJECT	I	TIME	u	$uU_{Na}$	$uU_{Cl}$	$uU_{HCO_3}$	$uU_{NH_3}$	$uU_K$	pH	$L_{H_2O}$
		min.								
KCl..... L. E.....	66.9	0 420	 7.91	 316	 594	 8.1	 24.6	 425	 6.10	 310
KCl..... D. S.....	92.7	0 420	 5.86	 375	 820	 1.8	 52.1	 516	 5.38	 -200
KCl..... B. W.....	117	0 420	 4.25	 162	 600	 4.3	 39.6	 524	 5.90	 560
KCl..... S. P.....	122	0 420	 5.64	 361	 812	 34.2	 21.4	 611	 6.88	 445
KCl..... G. N.....	142	0 420	 3.00	 144	 492	 3.6	 24.9	 436	 6.11	 221
KCl..... A. B.....	168	0 349	 4.60	 515	 667	 41.0	 19.8	 484	 6.61	 -580

TABLE 3

*Difference in plasma concentration between 7th hour and control period ( $A_7 - A_0$ )*

+ indicates an increase by the 7th hour, - indicates a decrease, and 0 indicates no significant change established in these experiments. These are overall effects and dilute solutions might have significantly different effects than concentrated ones.

SOLUTIONS	$(A_7 - A_0)$			
	Na	Cl	K	$HCO_3$
Water.....	0	0	0	0
NaCl (hypotonic).....	0	0	0	0
NaCl (hypertonic).....	0	+	-	0
$NaHCO_3$ .....	0	-	-	+
$KHCO_3$ .....	-	-	+	+
KCl.....	-	+	+	-
$NH_4Cl$ .....	-	+	0	-

Cl. Although  $U_K$  and  $I_K$  can be equal at concentrations above 70 m.eq./l., i.e., there may be no relative retention of K to water, the output of K is no longer able to keep up with the intake and the LIC is assumed to have been reached. This is implicit in the definition of isorrhea. While LIC solutions suffer no retention of solute relative to water, isorrhea, or equilibrium of output and intake should be attainable with them when the proper load is reached (Wolf, 1943; 1945a). Since solutions of KCl stronger than 70 m.eq./l. do not reach equilibrium of output and intake, that concentration is designated the

LIC<sup>2</sup>. Between 15 and 70 m.eq./l. all KCl solutions are readily isorrheic with respect to K<sup>3</sup>.

Table 3 indicates the gross effects of the tested solutions on the plasma concentration of the various ions.

Table 4 lists the velocity constants (rate of excretion per unit load) and the relative excretion rates of the various ions tested. Rates are arbitrarily based on the velocity constant of Na from NaCl, taken as unity, at given *I* values (interpolated). In addition to providing a basis for comparing the excretion rates of the different components, these relative excretion rates afford evidence

TABLE 4

*The velocity constant ( $\gamma$ ), or rate of excretion per unit load, averaged for the 6th and 7th hours, at *I* values (by interpolation) of 75 and 125 m.eq./l.*

The relative excretion rate is the ratio of the  $\gamma$  of an ion to the  $\gamma$  of Na from NaCl, the latter taken arbitrarily as unity. In section A the Cl of KCl and of NH<sub>4</sub>Cl was in negative load and  $\gamma$  was not computed. The relative excretion rates of water and Cl of NaCl may be found from data of Wolf (1943) for the dog and Wolf (1945a) for man.

<i>A (I = 75 m.eq./l.)</i>				<i>B (I = 125 m.eq./l.)</i>			
Substance	Ion	$\gamma$	Relative excretion rate	Substance	Ion	$\gamma$	Relative excretion rate
KCl	Cl			KCl	Cl	0.0163	27.2
NH <sub>4</sub> Cl	Cl			NH <sub>4</sub> Cl	Cl	0.00361	6.01
KCl	K	0.00690	9.20	KCl	K	0.00326	5.44
KHCO <sub>3</sub>	K	0.00425	5.66	KHCO <sub>3</sub>	HCO <sub>3</sub>	0.00239	3.98
NaHCO <sub>3</sub>	Na	0.00360	4.80	KHCO <sub>3</sub>	K	0.00230	3.84
KHCO <sub>3</sub>	HCO <sub>3</sub>	0.00240	3.20	NaHCO <sub>3</sub>	Na	0.00180	3.00
NaHCO <sub>3</sub>	HCO <sub>3</sub>	0.00150	2.00	NaHCO <sub>3</sub>	HCO <sub>3</sub>	0.00140	2.34
NaCl	Cl	0.00109	1.45	NaCl	Cl	0.00067	1.12
NaCl	Na	0.00075	1.00	NaCl	Na	0.00060	1.00
NH <sub>4</sub> Cl	NH <sub>4</sub>	0.00036	0.48	NH <sub>4</sub> Cl	NH <sub>4</sub>	0.00033	0.55

that no single ion uniquely determines the excretion of water or that of associated ions. To a large extent the behavior of a compound is determined by its specific

<sup>2</sup> The LIC is not necessarily the maximum urinary concentration (MUC) (Wolf, 1943). The MUC is the highest concentration attainable in the urine, when there may be retention of solute relative to water, or when a load of a solute may be accumulating excessively in the body. The MUC for K is over 200 m.eq./l. since values of this order have been found during these experiments. This contrasts with an LIC of 70 m.eq./l.

<sup>3</sup> Above the LIC the toxic action of K described by Keith et al. (1942) is evident. To the observations of these workers a few notes on several subjects drinking KCl solutions stronger than the LIC may be appended. Bradycardia gradually set in, reaching a base level of 50 to 60 beats per min. in about 3 hours (this occurred even with dilute solutions). By the 4th hour, restlessness, loquacity, or whimsey set in. Later a hesitancy in speech, difficulty in enunciation, subjective tightening of facial muscles, tiredness of jaw and leg muscles, and paresthesia of face and body developed. By the 5th hour in a subject taking 0.168 N KCl, eructation and nausea were marked. At the 349th minute, vomiting of several hundred cc. of brownish fluid brought rapid recovery from acute symptoms. An hour later strong leg cramps came on but soon passed off. In some subjects, diarrhea seemed imminent.

combination of ions. Thus K from  $\text{KHCO}_3$  is not excreted very differently from the Na of  $\text{NaHCO}_3$  (e.g., a ratio of 5.66/4.80) but the K from KCl is very different from the Na from NaCl (e.g., a ratio of 9.20/1.00). The Na from  $\text{NaHCO}_3$  can be excreted 4.80 times as fast as the Na from NaCl. From table 4B we see Cl excretion greater than that of K in KCl in the ratio of 27.2/5.44. Of special interest is the singularly low relative excretion rate of  $\text{NH}_3$ .

DISCUSSION. a. *Actual and theoretical u/i values with NaCl.* The fit of the actual values to the theoretical curve is better for hypertonic solutions given by vein than for the same solutions given orally. It was believed (Wolf, 1945a) that the poor absorption of some hypertonic solutions from the gut was responsible to a large extent for the discrepancy between actual and theoretical values.

The discrepancy between actual and theoretical values with intravenous infusions seems significant. From its nature the theoretical curve does not indicate what the actual values ought to be at any time, but simply what they would be if the kidneys maintained a constant, normal plasma concentration of Cl or Na, with or without change in extracellular volume, in the face of an infusion of salt solution. The magnitude of the discrepancy is, therefore, a measure of the change in two almost indissolubly combined factors, viz., plasma Na or Cl concentration, and distortion of the internal environment. The term distortion, discussed by Peters (1942) and Wolf (1943), refers to an imbalance of the normal relative volumes of the extracellular and/or intracellular volumes. In general the discrepancy between the actual and theoretical values is less with iso- and hypotonic solutions than with hypertonic ones. It appears that man more effectively is protected from dilution and distortion than from concentration and distortion. It is suggested that just as the maintenance of normal extracellular concentrations takes precedence over the regulation of extracellular volume, so may the regulation of intracellular volume take precedence over the regulation of extracellular volume, particularly with infusions of dilute solutions. Where theoretical and actual values agree by overlap in the isotonic range the equation describes the fact that although distortion is marked, the plasma concentration has suffered no substantial change.

It is not known whether the renal regulation of plasma Na or Cl concentration can occur independently of the regulation of intracellular volume under the conditions of these experiments.

b. *Urinary  $\text{NH}_3$  and systemic acid-base balance.* The rôle of the urinary  $\text{NH}_3$  in the base-economy, long discussed, has been elaborated by Gamble (1942) and others. It is supposed that the excretion of ammonia in the urine is essentially a protective response of the kidney enabling fixed base, chiefly Na, to be spared an excretory fate, and wasting. Briggs (1932, 1934, 1942) has brought together a series of experimental evidences to show that the renal ammonia mechanism probably has nothing to do with the regulation of the acid-base balance of the body. It is unnecessary to review all facts attesting each view. Briggs holds that  $\text{NH}_3$  excretion is stimulated by and serves to neutralize the acid residue left in the tubules, as a local, protective function. Peters (1935) has criticized Briggs' viewpoint and favored the classic concept.

Present experiments are believed to favor the Briggs hypothesis only in regard

to the negative rôle of  $\text{NH}_3$  excretion in the systemic acid-base balance of normal man, for the following reasons:

1.  $\text{NH}_3$  excretion is not specifically stimulated by  $\text{NaCl}$  infusions containing  $\text{Cl}$  in higher concentration than in plasma.

2. The unchanged excretion rate of  $\text{NH}_3$  following variously concentrated  $\text{NaCl}$  infusions, parallels the unchanged  $\text{pH}$  of the urine.

3. With no exceptions yet shown to be significant, the excretion of  $\text{NH}_3$  is found to be a function of urinary  $\text{pH}$ ; and no sudden or critical change in its excretion rate is found as the urine varies from alkaline to acid.

4. When acidosis is induced by  $\text{NH}_4\text{Cl}$  solutions, the increased rate of  $\text{Na}$  excretion with time far exceeds that of  $\text{NH}_3$  excretion (table 2) and it is apparent that fixed base is not being saved significantly. That considerable reduction in alkali reserve can be safely sustained may mean that the extent of systemic tolerance for acid intakes measures not only viability, but the insurance for subsequent attainment of normal acid-base balance through renal regulation. Protection of base by  $\text{NH}_3$  is frankly late. This is again seen in the low velocity constant for  $\text{NH}_3$  excretion.<sup>4</sup>

5. That it is not simply the fixed base concentration in plasma (as opposed to body content of fixed base) which influences  $\text{NH}_3$  excretion is evidenced by comparing the effects of  $\text{NH}_4\text{Cl}$  and  $\text{KHCO}_3$  solutions. Both bring about reductions of plasma  $\text{Na}$  concentration (with  $\text{KHCO}_3$ , although the positive load of  $\text{K}$  matches the negative load of  $\text{Na}$  (fig. 8), the concentration of plasma  $\text{Na}$  falls more than that of  $\text{K}$  rises)<sup>5</sup> but their effects on  $\text{NH}_3$  excretion are opposite. Plasma  $\text{Na}$  was not protected so efficiently during  $\text{NH}_4\text{Cl}$  or  $\text{KHCO}_3$  intakes, as during pure water intakes.<sup>6</sup>

Admittedly the evidence for the classic concept and for the Briggs hypothesis is circumstantial. No clearly probative demonstration has been brought forward in either case. There are inconsistencies in both viewpoints. Yet if the excretion of  $\text{NH}_3$  in normal man is regarded primarily as a function of urinary  $\text{pH}$ , no

<sup>4</sup> It is worth noting that although the velocity constant of  $\text{NH}_3$  is very small and therefore large changes in excretion rate are not quickly induced, there is no evidence that absolute changes in  $\text{NH}_3$  excretion do not occur essentially as rapidly in response to the appropriate stimulus as do changes in the excretion of any other substances examined.

<sup>5</sup> With  $\text{I}_{\text{NH}_4\text{Cl}} = 127 \text{ m.eq./l.}$ , plasma  $\text{Na}$  fell 6 m.eq./l. in 7 hours. With  $\text{I}_{\text{KHCO}_3} = 130 \text{ m.eq./l.}$ , plasma  $\text{Na}$  fell 4 m.eq./l. and  $\text{K}$  rose 2.2 m.eq./l. in 7 hours.

<sup>6</sup>  $\text{U}_{\text{Na}}$  during  $\text{NH}_4\text{Cl}$  or  $\text{KHCO}_3$  diuresis was of the order of 60 or 70 m.eq./l. with the stronger solutions. Were plasma  $\text{Na}$  protected completely, then from equation (1),

$$\frac{u}{i} = \frac{140 - 0}{140 - 60} = 1.75; \quad \frac{u}{i} = \frac{140 - 0}{140 - 70} = 2.00$$

Corrected for extra-renal water loss, these values respectively would be ca. 1.57 and 1.80. Actual  $u/i$  values never exceeded 1.7 and were usually of the order of 1.1.  $\text{U}_{\text{Na}}$  during water diuresis was ca. 14 m.eq./l. Were plasma  $\text{Na}$  protected completely, then from equation (1)

$$\frac{u}{i} = \frac{140 - 0}{140 - 14} = 1.11$$

Corrected for extra-renal water loss, this would be ca. 1.00. Actual  $u/i$  values were of the order of 0.97.



matter how that pH is effected, a good working hypothesis, parsimonious in assumptions, and substantially in accord with all facts, is obtained.

c. *Threshold of retention for K.* Properly no threshold of retention for K can be said to exist unless it is shown that urinary K concentration can be less than the concurrent plasma K concentration. Griffon's (1936) computation of a threshold for K is felt not to be admissible. Stewart and Rourke (1942) found urinary K values less than 1.4 m.eq./l. which constitutes presumptive evidence for a threshold of retention. In the present study, two subjects receiving a water intake (one oral, one intravenous, with glucose) showed at the 3rd and 4th hours, at the peak of the diureses, urinary K values of 3.6 to 4.1 m.eq./l. These may have been below the plasma concentration, further suggesting the existence of a threshold of retention.

However, in practically all short term experiments, since the threshold (in m.eq./l.) is so low, K acts as if it were a no-threshold substance. Thus for K the equation of steady state may be written

$$\frac{u}{i} = \frac{(A_T)_K - I_K}{(A_T)_K - U_K} = \frac{I_K}{U_K} \quad (2)$$

where  $A_T$  is assumed to be zero effectively; or

$$uU_K = iI_K \quad (3)$$

and this equilibrium (fig. 9) is easily attained with many other substances.

d. *Diuresis and ecuresis.* There are serious defects in concepts of diuresis based on "osmotic pressure" or on the "acidifying" nature of some diuretics (Shannon, 1938; Keith and Binger, 1935). Classification of diuretics on these bases, while expedient, is not always rational. Some diuretic actions regarded as "osmotic," e.g., urea or NaCl, are more properly referable to the LIC of these substances<sup>7</sup>. Thus equations (2) and (3) hold for many substances where it is seen that an increase in  $u$ , or a specific diuretic effect attending an increase of  $I$  above the LIC, is dependent on the  $U_{LIC}$  which may be relatively constant (i.e.,  $u$  is proportional to  $I$  or to  $iI$  when  $U$  is at the LIC). Substances which act in this way are "isorrheic diuretics."

Diuresis has been defined nicely as a temporary increase in the rate of urine formation. However, some diureses induce an absolute dehydration of the body to a water content lower than the pre-diuretic period. It is believed useful to regard diuretics as either *ecuretic*, if they bring about absolute dehydration, or *non-ecuretic*, if they do not bring about absolute dehydration. Thus mercurials, water, NaCl solutions below the MIC and NaCl solutions above the LIC, are often *ecuretic*. NaCl solutions between the MIC and the LIC are *non-ecuretic* diuretics. Isorrheic diuretics are specifically *ecuretic* (more so than water or saline solvent) when given so that the ratio of their intake to that of water is above their LIC.

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<sup>7</sup> NaCl solutions of increasing osmotic pressure (e.g., from 0 to 350 m.eq./l.) do not show correspondingly increasing diuretic effects (fig. 1). KCl solutions (e.g., from 0 to 100 m.eq./l.), however, are all strongly, almost equally diuretic. Specific action of ion combinations stands clearly apart from osmotic phenomena. The degree to which a given LIC depends on osmotic pressure is unknown.

Peters (1932) raised the interesting question as to whether K has any specific diuretic influence or whether diuresis after administration of K or any indifferent ion is not referable simply to restriction of NaCl. One may view this negative aspect (diuresis due to restriction of NaCl) as a positive one, i.e., diuresis due to relative excess of water. It is true that certain dilute solutions of KCl (or intakes of KCl and water such that the ratio of their respective intakes is low) cannot be distinguished from water (fig. 8) so far as their effects on the load of water remaining after a short time are concerned. But KCl solutions have a greater specific effect in removing Na, and some  $\text{HCO}_3$ , than does water, and in longer periods of adjustment, specific ecuretic effects of KCl may be demonstrable. In addition, KCl when administered considerably above its LIC, is very ecuretic although toxic effects enter the picture. And  $\text{KHCO}_3$  is more ecuretic than KCl, again revealing the difficulty in attempting to interpret completely the action of a compound from one of its components. Urea is specifically ecuretic only above its LIC (Wolf, 1943).

The acidifying nature of salts cannot consistently be regarded as primary in their diuretic function. Thus  $\text{KHCO}_3$  is more effective than  $\text{NH}_4\text{Cl}$ ;  $\text{NaHCO}_3$  is more effective than NaCl.

#### CONCLUSIONS

The renal responses of man to steady intakes of water with and without dissolved electrolytes (NaCl,  $\text{KHCO}_3$ ,  $\text{NaHCO}_3$ , KCl, and  $\text{NH}_4\text{Cl}$ ) have been studied. NaCl solutions were administered intravenously and all others orally, at 7 cc./min. for 7 hours.

1. Ratios of urinary output rate to intake rate for intravenous infusions of 7 hours' duration in man have been determined for NaCl solutions of 0 to 344 m.eq./l. concentration.

2. The minimal isorrheic concentration (MIC) for Na and Cl of NaCl and for K of KCl was ca. 15 m.eq./l. The limiting isorrheic concentration (LIC) for Na, Cl, and K, respectively, was ca. 290, 340, and 70 m.eq./l.

3. It is believed that man is more effectively protected against dilution and distortion of the internal environment than against concentration and distortion of the internal environment; and that the regulation of intracellular volume, like that of plasma concentration, may in some cases take precedence over the regulation of extracellular volume.

4. The pH of urine formed in the course of 7 hour steady intakes of variously concentrated solutions of NaCl,  $\text{KHCO}_3$ ,  $\text{NaHCO}_3$ , KCl,  $\text{NH}_4\text{Cl}$ , and of water has been determined. Alkaline tides were obtained with all but NaCl. Those of KCl and  $\text{NH}_4\text{Cl}$  were transient, the latter being relatively small and followed by increased acidification of the urine.

5. Hypertonic solutions of NaCl increase the excretion of K more than hypotonic solutions of NaCl.

6. The excretion of  $\text{NH}_3$  is almost independent of the concentration of NaCl infused.

7. Curves relating the excretion rates of  $\text{NH}_3$  and  $\text{HCO}_3$  to urinary pH have been determined. The rates were equal at ca. pH 6.8.

8. The 7th hour retention (load) of water, Na, K,  $\text{NH}_3$ , Cl, and  $\text{HCO}_3$  after intakes of NaCl,  $\text{KHCO}_3$ ,  $\text{NaHCO}_3$ , KCl, and  $\text{NH}_4\text{Cl}$  solutions, and water, is given; also the gross changes in plasma concentrations of the ions named.

9. K behaves in many experiments of short duration in normal man as a no-threshold substance. It is believed, however, actually to have a low but undetermined threshold of retention.

10. The velocity constants and the relative excretion rates of all ions studied were determined. The excretion rate of water, or of a load of a given ion from an infused compound depended in part on the electrically associated ion of the compound.

11. Evidence favoring in part the Briggs hypothesis of  $\text{NH}_3$  excretion is presented. It is believed that the excretion of  $\text{NH}_3$  in normal man does not play a primary rôle in the renal regulation of systemic acid-base balance.  $\text{NH}_3$  excretion was more highly correlated with urinary pH, however effected, than with any other single variable tested.

12. Classifications of diuretics as "osmotic" or "acidifying" is believed inadequate for modern renal physiology. The term "isorrheic diuretic" is suggested for those substances whose diuretic effect is referable to the inability of the kidney to exceed a limiting isorrheic concentration. Diuretics are classified as *ecuretic* when they bring about an absolute dehydration of the body and *non-ecuretic* when they do not.

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# THE MECHANISM OF PROLONGED FLUORESCEIN CIRCULATION TIME IN EXPERIMENTAL TRAUMATIC SHOCK<sup>1,2</sup>

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Evidence indicating that changes in fluorescein circulation time can be used as a simple and reliable prognostic index of the condition of dogs after muscle trauma has been previously reported (1, 2). During the period of incipient shock the fluorescein circulation time shows a gradual increase in animals which eventually die. In contrast to this progressive increase, simultaneously determined cyanide circulation time increases to a value which is maintained until the mean blood pressure falls below 50 mm. Hg when it undergoes a further increase. We have suggested that the discrepancy between the results obtained with the above circulation time methods may be explained by the fact that the fluorescein measurement is influenced by the condition of the minute systemic vessels; that a gradual progressive increase in circulation time indicates a vasoconstriction and failure of the peripheral circulation in traumatic shock. The present investigation is concerned with the analysis and extension of these observations.

**METHODS.** Upper thoracic sympathectomies were performed on 11 dogs under intravenous nembutal anesthesia (35 mgm. per kilogram of body weight). One upper thoracic sympathetic chain, including the stellate ganglion and the upper 4 or 5 thoracic ganglia, was removed through an incision in the third intercostal space (3). In seven of the animals, a second operation was performed on the contralateral side after a period of convalescence of 7 to 10 days. The trauma experiments on these animals were carried out 5 to 7 weeks after the first operation at a time when the animals were in good health, showing a slight gain or at least a return to their pre-operative weight.

The detailed procedure of our trauma experiments was reported elsewhere (2) and the method adopted for producing shock was that described by Gregersen and Root (4). On the day before the trauma experiment a control circulation time was taken, and the plasma volume was determined by the dye (T-1824) dilution method. The total blood volume was calculated from the measured plasma volume and the hemotocrit value (see 5). On the following morning

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<sup>2</sup> A preliminary report of this work appeared in *Fed. Proc.* 4: 55, 1945.

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control determinations of the rectal temperature, heart rate and mean blood pressure (by femoral arterial puncture) were made. Under ether anesthesia both thighs were then contused. The clinical course of the animal was followed and the above observations made at regular intervals. The plasma volume determination was repeated about one hour after the trauma.

The fluorescein circulation time was determined by injecting rapidly through a 20 gauge needle 1 cc. of 10 per cent fluorescein solution. Injection was made into the femoral vein and the determination was carried out in a dark room. The time taken for the fluorescent color to appear in the conjunctival mucosa observed under ultraviolet light was recorded as the circulation time (6). In a few animals the cyanide circulation time was also determined using 1 cc. of a 0.1 per cent solution of sodium cyanide (7). The end-point of the latter method is the first appearance of a forceful inspiration or gasp.

TABLE 1

*Comparison of fluorescein (F) and cyanide (CN) circulation times in traumatized dogs*

DOG NUM- BER	SEX	BODY WEIGHT	BLOOD VOLUME		CIRCULATION TIME IN SECONDS							DURATION OF SURVIVAL AND OTHER REMARKS
			Per cent reduc- tion	Resid- ual vol- ume	Control	Number of hours after trauma						
						0-1	1-2	2-3	3-4	4-5	5-6	
		kgm.		cc./ kgm.								
N2	♀	11.6	29	75	(F) 15 (CN) 14		37 22	37 20	40 24	53 27	71 23	Died shortly after last (F) determination
N3	♀	7.1	21	61	(F) 12 (CN) 11	30 20	32 23	37 21	42 23	92 No effect		Died during last (CN) determination
N4	♀	6.4	42	67	(F) 13 (CN) 11	23 20	30 20	30 24	70 24		35	Died shortly after last (CN) determination

Throughout the period of observation the animal was restrained on a comfortable animal board until death occurred or for a period of 6 hours after which it was returned to the cage.

**RESULTS.** Experiments presented in table 1 demonstrate that if animals are traumatized severely and a fatal shock results, the circulation time as determined by the fluorescein method shows a slow but definite prolongation and reaches 70 seconds or more as the animals approach death. The simultaneously determined cyanide time increases to a plateau value and is usually not further increased until the blood pressure of the animal falls below 50 mm. Hg. In dog N4 (table 1) the cyanide circulation time remained at 20 to 24 seconds for almost 4 hours and increased only to 35 seconds 10 minutes before death. Over the same period, the fluorescein time gradually increased to 70 seconds. In dog N3 the final cyanide injection was made when the animal was showing a terminal hyperpnea and no definite end-point was perceived.

Of 7 dogs with bilateral upper thoracic sympathectomies, 6 succumbed following trauma. Their average control heart rate was 81 beats per minute (with a standard error of 5) as against an average heart rate of 105 beats per minute (S. E. 3) in a group of 57 normal dogs. The control mean blood pressures and fluorescein circulation times of these animals were within normal range. Following severe muscle trauma, the average heart rate increased and remained at 149 beats per minute (S. E. 10), showing no terminal rise before death. These animals showed a plateau in fluorescein circulation time of 23 to 29 seconds for several hours with an abrupt rise one hour before death (table 2). This is in contrast to the behavior of fluorescein circulation time in the normal traumatized animal where approaching death is indicated by a gradual and progressive increase in fluorescein time (see fig. 1).

TABLE 2

*Repeated determinations of fluorescein circulation time in traumatized dogs with both upper thoracic sympathetic chains removed*

DOG NUM- BER	SEX	BODY WEIGHT	BLOOD VOLUME		FLUORESCIN TIME IN SECONDS						DURATION OF SURVIVAL AND OTHER REMARKS
			Per cent reduc- tion	Resid- ual vol- ume	Con- trol	Number of hours after trauma					
						1-2	2-3	3-4	4-5	5-6	
		<i>kgm.</i>		<i>cc./ kgm.</i>							
1	♀	10.7	43	59	15	26	26	26	51	51	6 hrs. died shortly after last determination
2	♀	8.8	44	66	15	29	28	68			4.3 hrs. died shortly after last determination
3	♀	9.0	30	67	12	26	28		26	52	6 hrs. died immediately after last determination
4	♂	11.9	36	58	10	23		25		38	5.4 hrs.
5	♀	7.8	32	65	11	28	45				2.4 hrs. died shortly after last determination
6	♂	13.6	41	53	14	29					2.4 hrs. died 1.3 hrs. after last determination
7	♀	13.3	28	74	11	27		27	23	15	Recovered

In 4 dogs with unilateral upper thoracic sympathectomies there was no appreciable deviation of heart rate or mean blood pressure from normal. The control fluorescein circulation time was identical on the normal and operated sides. The skin temperature of the ear on the operated side was only questionably higher and the pupillary sphincter was slightly narrower than that of the normal side. The clinical manifestations of these dogs following severe muscle trauma were the same as those in normal dogs subjected to fatal traumatic shock. During the time such animals were in shock, however, the difference in skin temperature of the two ears and in the size of the pupils became much more prominent. The circulation time following trauma was usually not as prolonged on the operated as on the normal side (table 3).

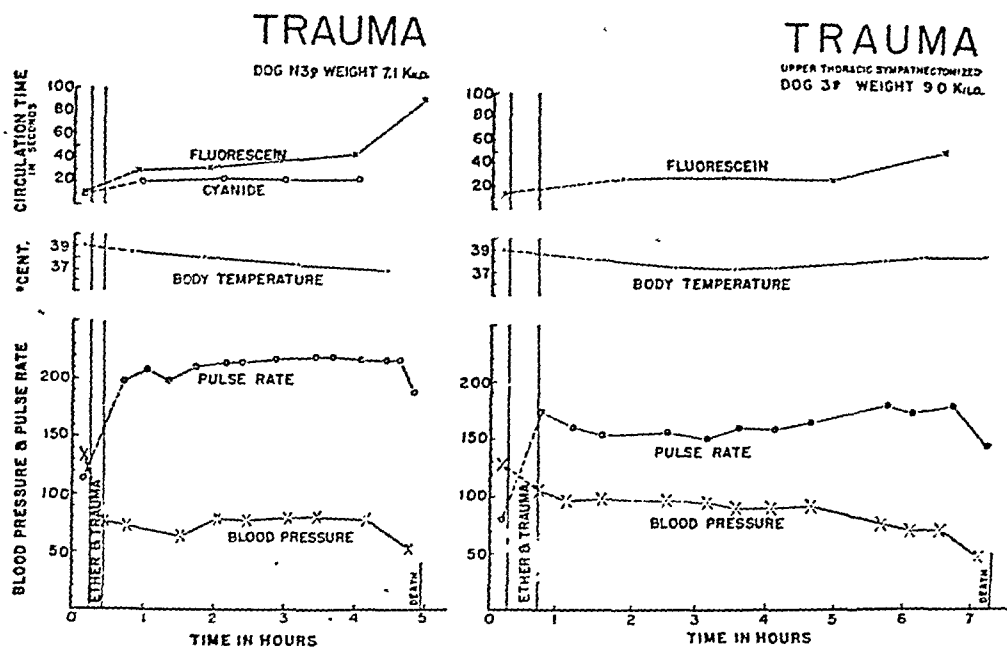


Fig. 1. Comparison of the clinical manifestations (body temperature, pulse rate and mean blood pressure) and fluorescein circulation time in normal dog N3 and upper thoracic sympathectomized dog 3 in traumatic shock. In the normal dog, the fluorescein circulation time showed gradual prolongation and in the sympathectomized dog it was maintained at an increased level in the early stage of shock. Repeated determinations of cyanide circulation time were also made on dog N3. No cyanide response could be obtained just before death. Data on dog N3 has appeared in a previous publication (2).

TABLE 3

Comparison of fluorescein circulation time between normal (N) and sympathectomized (S) sides in traumatized dogs

DOG NUM- BER	SEX	BODY WEIGHT	BLOOD VOLUME		FLUORESCIN TIME IN SECONDS					DURATION OF SURVIVAL AND OTHER REMARKS	
			Per cent reduc- tion	Resid- ual vol- ume	Control	Number of hours after trauma					
						1-2	2-3	3-4	4-5		5-6
		kgm.		cc./ kgm.							
8	♀	14.6	32	70	(N) 16 (S) 16	35 30	46 39				3.6 hrs.
9	♀	8.3	40	64	(N) 10 (S) 10	44 32					2.8 hrs.
10	♂	8.8	25	65	(N) 11 (S) 11	45 39					2.2 hrs.
11	♀	11.6	29	56	(N) 13 (S) 13		33 33		33 23	206? 200?	5.5 hrs. Died imme- diately after last de- termination

DISCUSSION. In any circulation time test one determines the speed of blood flow in the vascular system from a selected point of injection to the point of observation. The result of a particular test does not necessarily reflect the condition of the entire vascular system since the estimation is made in only a portion of the circulatory apparatus and different conditions may prevail in other circuits. For instance, the cyanide time measures the linear velocity in that portion of the vascular system from the femoral vein via the pulmonary circulation to the systemic arterial side where chemoreceptors are located in the region of aortic arch and the bifurcation of the common carotid arteries. Inasmuch as the time taken for the reflex action to effect an abrupt stimulation is short (1 to 2 sec.), and the reaction time is not increased in shock (8), any prolongation of the cyanide time in traumatic shock must be interpreted as an impaired venous and/or pulmonary circulation (7). In the fluorescein determination, however, the end-point is not taken when the fluorescent color reaches the smaller arteries and arterioles, but is taken only after the arrival of the test substance in the capillaries of the conjunctival mucosa. In an animal with no background fluorescence, the difference in these arrival times can be easily seen. Whereas in the normal dog the mucosa becomes rapidly fluorescent within a fraction of a second after the arrival of fluorescein in the arterioles, in the traumatized dogs the maximum hue develops more slowly. It is evident that the fluorescein method used in this way not only measures the cyanide circuit, but also measures a portion of the systemic arterial tree including the peripheral capillary bed.

Since the control fluorescein circulation time is only one or at most two seconds longer than the control cyanide time, the increasing difference between the two methods in shock must be accounted for by some factor other than the greater distance involved in the measurement by fluorescein. The discrepancy between the two methods indicates a progressive impairment of the peripheral systemic circulatory apparatus in traumatic shock which is revealed only by the fluorescein method.

It is well known that animals develop a generalized vaso-constriction following muscle trauma or blood loss (9). This compensatory process is mediated through the sympathetic nervous system (10). Extreme narrowing of the caliber of the arterial branches reduces the blood flow, increases the degree of tissue hypoxia, and contributes to the failure of peripheral circulation. It is reasonable, therefore, to expect that in the sympathectomized vessels this extreme constriction will not develop or that at least it will not become progressively more intense throughout the course of shock.

It is of interest to note that during the stage of impending shock the ears of the bilaterally sympathectomized animals were warm and their pupils were not widely dilated. There was no evidence of depression of the sensorium until the mean blood pressure had fallen below 60 mm. Hg, whereas in the traumatized normal animal central nervous depression appears early in the



course of shock (4, 11). This difference in the double sympathectomized animal is presumably due to the fact that cerebral vessels are not so intensely constricted since they are not receiving sympathetic nervous impulses (12). The contrast in the two conditions is grossly apparent in the traumatized animal in which a unilateral sympathectomy has been previously performed. Here the differences in skin temperature of the two ears and in the size of the pupillary sphincters are striking, the ear being warmer and the pupil smaller on the operated side than the corresponding structures on the normal side.

The change in heart rate of the bilaterally sympathectomized dogs in shock is also decidedly different from that in shocked normal animals. Following muscle trauma under ether anesthesia, normal animals not infrequently develop an immediate tachycardia of over 200 beats per minute which, in many instances, continues throughout the period of shock. Such a tachycardia following trauma is probably the result of a combination of mechanisms; 1, augmentation of the sympathetic cardioaccelerator mechanism; 2, the influence of liberated sympathomimetic substances on the heart, and 3, a decrease in the vagal inhibitory effect or an increase of the vagal accelerator effect on the heart. It is well known that ether itself accelerates the heart in the normal animal through these same mechanisms (13) and consequently the high heart rate immediately following the traumatic procedure may be in part the result of residual ether.<sup>5</sup> In our bilaterally thoracic sympathectomized dogs the heart rate following muscle trauma increases to an average of 149 beats per minute. None of the 6 animals that died developed a terminal heart rate above 180 beats per minute. These rates differ considerably from those of normal shocked dogs which show a much higher average heart rate throughout the shocked period.

The differences in various clinical signs between traumatic and simple hemorrhagic shock will be pointed out elsewhere (11). These differences are related to the amount of sympathetic discharge operating through an afferent nervous factor in traumatic shock. What information the fluorescein circulation time can add to this concept of differences in sympathetic activity between the two types of shock would be helpful not only for the solution of the problems, but also for the reliability of circulation time as a prognostic index in hemorrhagic shock. For this reason a few experiments were carried out in order to study the circulation time by the fluorescein and cyanide methods in hemorrhaged animals. The detailed procedure of hemorrhage itself is reported elsewhere (11). The results on the circulation time in such animals are shown in table 4. All 6 of the animals suffering from fatal hemorrhagic shock had a low fluorescein circulation time as compared with determinations in animals of the traumatic

<sup>5</sup> In a group of 16 chronic spinal dogs (sectioned at T<sub>10-12</sub>) (14), 10 were traumatized in the usual manner without ether anesthesia. The heart rate immediately following the procedure ranged from 108 to 180 beats per minute (average 132 with a standard error of 7). The remaining 6 animals were given ether during the traumatic procedure. The heart rate in this group was increased to an average of 191 beats per minute (range 180 to 210, S. E. 5) which in most cases lasted only a short time and was followed by a drop to 179 beats per minute (range 175 to 190, S. E. 3). The increase in heart rate immediately following trauma is definitely greater if ether is used as an anesthetic.

series which died in 3 or 4 hours (2). Indeed, several dogs show a fluorescein circulation time of less than 20 seconds when death is already imminent. This difference in circulation times cannot be explained on the basis of not having reduced the circulating volume sufficiently since in this group of animals the reduction in blood volume (38 to 46 per cent) is very severe, nor can the difference be attributed to the use of ether anesthesia during trauma since ether was also given during hemorrhage in order to make the two series comparable (11). The conclusion is inescapable, therefore, that the shorter fluorescein circulation time in fatal hemorrhage as compared with trauma is an indication of a relatively lower degree of peripheral vaso-constriction.<sup>6</sup>

TABLE 4

*Repeated determinations of fluorescein (F) and cyanide (CN) circulation time in normal dogs before and after simple hemorrhage*

DOG NUM- BER	SEX	BODY WEIGHT	BLOOD VOLUME		CIRCULATION TIME IN SECONDS					DURATION OF SURVIVAL
			Per cent reduction	Residual volume	Control	Number of hours after hemorrhage				
						1-2	2-3	3-4	4-5	
		<i>kgm.</i>		<i>cc./kgm.</i>						
12	♀	8.4	42	58	(F) 9	17	16	55		4.1 hrs.
13	♂	9.3	40	59	(F) 14	20	35			3.0 hrs.
14	♂	9.7	38	64	(F) 12 (CN) 11	18	22 19			3.8 hrs.
15	♂	9.3	39	71	(F) 13 (CN) 12	23 15	27 18		36 23	4.9 hrs.
16	♂	7.2	41	55	(F) 11 (CN) 9	27 15	39 16			3.9 hrs.
17	♀	9.0	46	58	(F) 11 (CN) 10	29 23	34 22			3.0 hrs.

The cyanide circulation time throughout the course of hemorrhagic shock is also shorter than that in traumatic shock. In fact, several animals show only a slight prolongation after a fatal hemorrhage (table 4). The discrepancy in circulation times following hemorrhage and muscle trauma in this case can only be ascribed to changes in the pulmonary vascular bed. Although the rôle of this system is not clearly understood in shock, it is known that the pulmonary vessels are innervated by the autonomic nervous system and that vaso-constriction is obtained on excitation of the sympathetic nerves (16). Our evidence from measurements of both fluorescein and cyanide circulation times is highly

<sup>6</sup> In agreement with this is the finding that the calculated peripheral resistance is higher in traumatized animals than in those in which the blood volume is reduced by hemorrhage (15).

suggestive that in traumatic shock the vaso-constriction is very intense and generalized, and may include the pulmonary circuit.

#### SUMMARY AND CONCLUSIONS

The fluorescein circulation time in normal dogs suffering fatal muscle trauma is progressively prolonged. Simultaneously determined cyanide circulation times do not show corresponding increases but instead show plateaus at increased levels. On the other hand, in dogs with bilateral upper thoracic sympathectomies the fluorescein circulation time following trauma behaves as does the cyanide circulation time in the normal traumatized animal.

These facts indicate that the mechanism of prolongation of the fluorescein circulation time in the normal dog in traumatic shock is associated with an increased activity of the sympathetic nervous system particularly upon the peripheral portion of the vascular tree.

The fact that neither the fluorescein nor the cyanide circulation times are as greatly prolonged following fatal hemorrhage as following fatal muscle trauma with a comparable blood volume reduction indicates that the sympathetic nervous system is not nearly so active throughout the course following hemorrhage as it is following muscle trauma.

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# RELATION OF MATERNAL BLOOD-FLOW WITHIN THE UTERUS TO CHANGE IN SHAPE AND SIZE OF THE CONCEPTUS DURING PREGNANCY; PHYSIOLOGICAL BASIS OF UTERINE ACCOMMODATION

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Few observations have been made on the rate of blood-flow through the uterus at different periods of pregnancy (Barcroft, Herkel and Hill, 1933; cf. Reynolds, 1939). Such observations as have been made pertain to the total blood-flow for the whole organ of litter-bearing animals. Determinations of total blood-flow for the whole organ neglect the fact that the uterine tissues about distended spheroidal conceptuses during the middle period of pregnancy are subjected to increasing tension resulting from the continually increasing size of the conceptus. On the other hand, other parts of the uterus lying between the implantation sites are subjected to less tension, and this should favor a channeling of blood through such areas in preference to those parts that are under greater local tension. The existing observations on blood-flow through the uterus further neglect the fact that local tension on the uterine wall alters according to the changes in size and shape of the uterus during pregnancy (Reynolds, 1946). It follows, therefore, that data pertaining to the conditions affecting the local flow of maternal blood into the vicinity of the conceptus have not, till now, been reported in the literature.

The importance of knowing the local circulatory conditions about the conceptus lies in the fact that fetal growth depends upon an adequate transfer of materials across the placenta. If the transfer of substances from the mother to the fetus is influenced by the changing pattern of uterine tissue resistance to the flow of blood through the uterus as it changes in size and shape, then the curve of fetal growth will reflect this influence. If conditions tending to limit an adequate flow of blood to the vicinity of the conceptus should become extreme or prolonged, fetal death might occur. Accordingly, the data reported in this study add to our appreciation of the physiological factors associated with fetal growth and welfare.

**METHODS AND MATERIALS.** *General.* The rate of blood-flow through the uterus was determined by measuring the circulation-time from the lateral uterine vein within the uterine wall, or other site of choice, to the maternal carotid body. This was accomplished by injection of small volumes (0.01 to 0.35 cc.) of sodium cyanide (2 mgm. per cc.). A tuberculin syringe was used for all injections. In addition to injection of the cyanide into the uterine vein at the site of maximum uterine distention around the conceptus, injections were made at the following sites: *a*, the lateral uterine vein at interconceptus sites; *b*, the lateral uterine vein in unoccupied and undistended portions of a gravid uterus, well removed

from a conceptus site; *c*, the lateral uterine vein of a non-gravid (sterile) uterine horn in a unilateral pregnancy; *d*, the lateral uterine vein at sites of resorbing fetuses; *e*, the femoral vein. In this way it was possible to judge the circulation-rate of blood locally within different parts of the genital tract, and in the somatic circulation as well.

Injections into the lateral uterine vein were made through a 27-gauge needle retained by a small clip. Between injections an exceedingly slow drip of physiological saline was maintained through the side-arm of an adapter. The uterus was kept within the abdominal cavity, which was closed by clamps after preliminary operative procedures were over.

The use of sodium cyanide for estimation of circulation times is advantageous, because it may be used repeatedly in threshold doses, and because the respiratory response to stimulation of chemoreceptors in the carotid body is definite and reproducible.

*Respiratory responses.* The respiratory response to sodium cyanide in small amounts is characterized by a transient change in the pattern of breathing. This may involve hyperpnea, polypnea, an inspiratory gasp, or any combination of these. Threshold responses were considered to be elicited when only one, or possibly two of the three responses occurred after a single injection. The duration of such a response was usually less than a quarter of a minute. Experience proved that when small amounts of sodium cyanide (6 to 100 micrograms per kgm. of body weight) were used, injections could be made at intervals of ten minutes without noticeable alteration in respiratory rate or pattern. When larger amounts (150–400 micrograms per kgm. of body weight) were used in some instances, fifteen, to twenty minutes were allowed to elapse between injections.

An essential condition for these experiments was that the animals be lightly anesthetized with an anesthetic which maintained good cardiovascular reflexes. Dial-Urethane (Ciba) in a dose of 0.4 cc. intravenously per kilogram of body weight was used. Operative procedures were carried out with supplemental ether anesthesia. The rabbits were covered with several layers of thick towelling. Body temperature (vaginal) usually fell about three degrees Fahrenheit during any series of observations which lasted about four to five hours.

*Animal selection and preparation.* Rabbits were used because of the ease with which pregnancy could be obtained and timed. Only rabbits pregnant for the first time were used. Moreover, the changes in shape and size of each conceptus which take place have been well-studied in relation to uterine growth (Reynolds, 1939; 1946) and to the change in size of the products of conception (Hammond, 1927). For orientation, the essential features of this require re-stating.

From the time of implantation until about the twenty-second day, the conceptus is spheroidal. It consists mostly of amniotic fluid. The radii increase to a maximum size of about 18 to 20 mm. During this period, the uterine tissues hypertrophy. Between the twentieth and twenty-fourth days the conceptus undergoes conversion from a spheroidal to a cylindrical shape. This is most definite when two or more conceptus sites "merge" (although they are, of course, separated by their membranes) but elongation occurs even when one conceptus is involved. The cause of the change is twofold (see Reynolds, 1946): *a*, there is a decrease in the quantity of amniotic fluid in which the fetus has floated until this time, and *b*, there is continued growth of the fetus so that it comes to lie against the wall of the uterus. At this time the fetus weighs about one-twentieth of its weight at birth nine or ten days later. After the twenty-fourth day, all enlargement of the conceptus is by growth in a *longitudinal* direction, without further appreciable increase in diameter. Uterine growth (increase in weight) is virtually nil at this time. The principal features of this series of changes are shown in the schematic conceptus-shapes about the dots of curve A in figure 1.

The hydrostatic basis of the conversion and subsequent elongation with fetal growth is discussed elsewhere (Reynolds, 1946). Most rapid transfer of substances across the placenta takes place (Flexner and Pohl, 1941) and most rapid fetal growth occurs (Hammond, 1935) during this final phase of uterine accommodation of the products of conception (period of uterine stretching).

*Procedures.* The experimental procedures employed in this study were as follows: After induction of anesthesia, the left femoral vein was exposed; a low mid-line incision was made but clamped shut in the lower abdomen; an intrapleural trocar was inserted into the intrapleural cavity through a low intercostal stab incision. Respiratory movements were recorded by tambour. Time in second intervals was recorded, along with the period of injection of the cyanide.

A series of five to nine successive threshold circulation-time responses were recorded from the femoral vein. The same procedure was repeated in the lateral uterine vein in whatever conceptus presented itself as the abdominal incision was opened for insertion of the needle, and closed again, after the needle was in place. Subsequently, determinations were made at other conceptus-sites, at interconceptus sites, at undistended portions of the uterus, or in non-gravid uterine horns as mentioned above. In every case at least five threshold determinations were made, or more if feasible. The venous network through which the sodium cyanide passed was judged by observation of the areas into which physiological saline passed when it was injected.

Following these determinations, measurements of the mesometrial-antimesometrial and of the transverse diameters of each conceptus were made. Vascular injections of Prussian blue were made through the aorta, and photographs of the uteri were made to show the distribution of dye for the purpose of correlation with the circulation data. These, along with a study of blood-vascular injection-corrosion preparations of vinyl acetate will comprise a separate report to be made elsewhere.

*Expression of results.* Blood-flow rates were expressed as the reciprocal of the circulation time. Thus a coefficient of the rate of blood-flow was obtained. This device was necessary, since on the twenty-second day of pregnancy twenty-two of fifty-eight injections of threshold or suprathreshold doses of sodium cyanide at conceptus sites yielded negative results, i.e., there was practically no local venous flow from the uterine wall. It also serves to show on the graph (fig. 1) faster circulation rates in a higher position than slower circulation rates.

The data were analyzed statistically for probable significance of different features of the various curves and groups of data obtained. Analyses were made for every day of pregnancy studied, the first (or non-pregnant state); the twelfth; the sixteenth; the twentieth; the twenty-second; the twenty-fourth; and the twenty-eighth; and for every site from which data were obtained. The mean, the standard deviation and the T-factor were calculated.

The probability that any two means for which T was computed could come from the same set of data was determined by reference to the P-table of Snedecor given in Treloar (1935). In this, all values of 0.05 or less are considered significant, showing that there is 1:20 chance or less that the two means compared could come from the same family of data.

**RESULTS. Data.** In this study fifty-four rabbits were used with a total of eight hundred and fifty-four observations of respiratory responses, distributed as shown in table 1. Means and standard deviations of the coefficients of the various blood-flow rates are given here, also.

The relation of the results of this study to each other, summarized in table 1, is shown, for the most part, in figure 1. The following comments may be made concerning their probable significance:

*Femoral-carotid body blood-flow.* (Curve B, fig. 1.) The decrease in rate of blood-flow shown in curve B between the first and sixteenth day is significant:

there is a 1:5,000 probability ( $P = 0.0002$ ) that the two means are from the same group of data. Similarly, the increase in blood-flow rate between days sixteen and twenty-two is significant to the extent of 1:700 ( $P = 0.0014$ ) and

TABLE 1

*Blood-flow rate coefficients  $\left(\frac{1}{(\text{circulation time})}\right)$  from the lateral uterine vein (in the uterine wall) to the maternal carotid body, and from the femoral vein to the carotid body*

Sodium cyanide used (concentration = 2 mgm. per cc.; dose, 0.06-0.400 mgm. per kgm. of body weight). Threshold respiratory response consisted of one or two of the following: hyperpnea, polypnea, inspiratory gasp.

DAY OF PREGNANCY	SITE OF INJECTION	NUMBER OF RABBITS	NO. OF DETERMINATIONS (n)	AVERAGE COEFF. OF BLOOD FLOW ( $\bar{x}$ )	STANDARD DEVIATION ( $\sigma$ )
1	Femoral vein	11	73	0.150	$\pm 0.026$
	Non-gravid uterus	13	81	0.118	$\pm 0.020$
12	Femoral vein	7	39	0.134	$\pm 0.027$
	Non-gravid uterus	7	30	0.108	$\pm 0.030$
	Spherical conceptus	7	41	0.087	$\pm 0.027$
16	Femoral vein	7	44	0.127	$\pm 0.031$
	Non-gravid uterus	6	30	0.112	$\pm 0.024$
	Spherical conceptus	7	46	0.079	$\pm 0.020$
20	Femoral vein	6	32	0.146	$\pm 0.027$
	Non-gravid uterus	6	36	0.112	$\pm 0.024$
	Spherical conceptus	8	57	0.071	$\pm 0.017$
22	Femoral vein	6	34	0.153	$\pm 0.022$
	Non-gravid uterus	5	18	0.106	$\pm 0.019$
	Inter-conceptus site	2	7	0.099	$\pm 0.025$
	Gravid uterus: cylinders	3	13	0.067	$\pm 0.030$
	spheres	13	58	0.030	$\pm 0.029$
24	Femoral vein	6	34	0.140	$\pm 0.030$
	Non-gravid uterus	5	29	0.110	$\pm 0.012$
	Gravid uterus: cylinder	6	41	0.090	$\pm 0.022$
	resorbing fetus	1	6	0.070	$\pm 0.020$
28	Femoral vein	8	43	0.132	$\pm 0.019$
	Non-gravid uterus	4	16	0.079	$\pm 0.028$
	Gravid uterus, cylinder	7	46	0.075	$\pm 0.024$

the subsequent reduction in systemic circulation rate by the twenty-eighth day (as represented by femoral to carotid body circulation rates) is highly significant ( $P = < 0.0002$ , or  $< 1:5,000$ ).

It therefore follows that in the first half of pregnancy the general level of the circulation is reduced, if the femoral-carotid rate is an adequate criterion of this.

There is then a progressive, transient rise to a normal level on the twenty-second day, and then a fall to a low level on the twenty-eighth day. A decrease in systemic blood pressure during "late pregnancy" in rabbits has been reported (Corbit, 1941) without special reference to the twenty-second day.

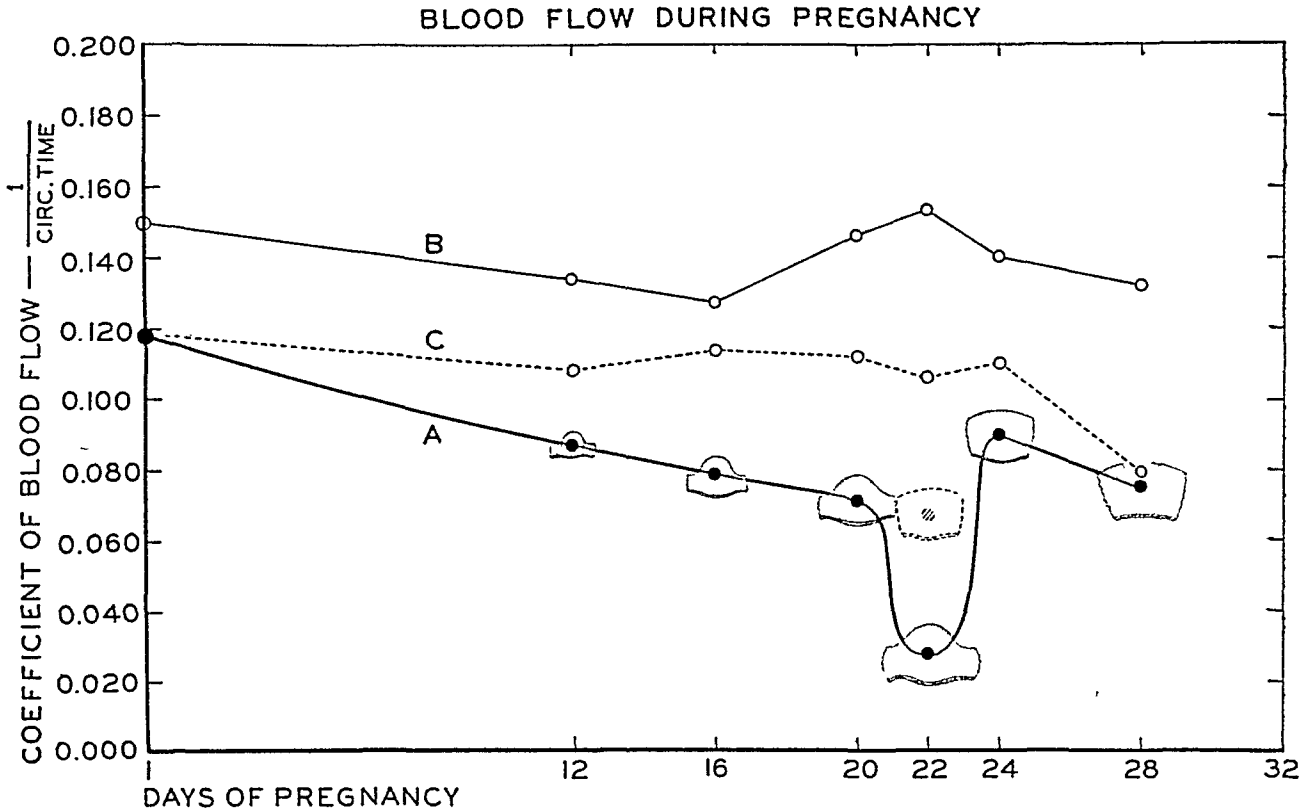


Fig. 1. Effect of shape and size of conceptus on the local flow of maternal blood in the uterine wall. Curve A shows that, as the radius of each spherical conceptus increases, there is a progressive diminution in rate of blood flow  $\left(\frac{1}{(\text{circulation time})}\right)$  of NaCN injected into the lateral uterine vein to the maternal carotid body. At a certain maximal radius, critical for each conceptus, a marked reduction in circulation-rate occurs, and this is relieved by rapid conversion from the spherical to the cylindrical shape. This takes place between the twentieth and twenty-fourth days. These changes are not found in the contralateral, non-pregnant horn in rabbits which are unilaterally pregnant (curve C), and it is in an opposite direction to the blood-flow in the systemic circulation, as judged by femoral vein-carotid body blood flow rates (curve B). The dotted, cylindrically shaped conceptus shown on the twenty-second day of pregnancy indicates blood-flow in a few conceptuses which had elongated by the twenty-second day of pregnancy, although other conceptuses in the same uteri were still spherical and gave evidence of very sluggish blood-flow (see text).

*Gravid uterus.* (Curve A.) Here, it will be seen there is a progressive decrease in circulation-rate between the first and twentieth day of pregnancy. The probability that the means for the first, and the twenty-second days of pregnancy could be from the same family of data is less than 1:10,000 ( $P = <0.0001$ ). The probability that the means for the twentieth and twenty-second days could be from the same groups is also of the same order (1:10,000;  $P =$



$<0.0001$ ). The similar probability for the means of the twenty-second and twenty-fourth days is less than 1:700 ( $P = <0.0014$ ). The return of blood-flow to a higher level on the twenty-fourth day than it was on the twenty-second day is highly significant; the means of these days stand a 1:5,000 ( $P = 0.0002$ ) chance of being from the same group of data.

It is clear, therefore, that throughout the first two-thirds of pregnancy, a steady and progressive fall in blood-flow rate takes place in the local circulation about the spherical conceptus. As the sphere attains a maximum diameter, a sudden, transient decrease in local blood-flow takes place. This is relieved,—in fact more than offset,—by conversion from the spheroidal to the cylindrical shape. Recovery of the circulation-rate takes place to an extent equal to that found about the twelfth day of pregnancy. The importance of this restoration of blood-flow, which coincides with a rapid upswing in the curve of fetal growth (cf. Reynolds, 1939), has been discussed in a recent paper dealing with the hydrostatic relations of hollow spherical and cylindrical elastic bodies (Reynolds, 1946).

*Proof of the relation of shape of conceptus to local blood-flow.* (See curve A.) Three rare opportunities presented themselves in which spherical conceptuses were found in the same uterine cornua with two or more which had already converted to the cylindrical form (see fig. 2 in paper by Reynolds, 1946). Blood-flow rates were determined in both the cylinders and spheres in which fetal size, placental size, distance between site of injection and the carotid body, and all maternal factors but local uterine shape were similar. The difference in circulation-rate is shown on curve A, day twenty-two, of figure 1. The probability that these two mean values could come from the same body of data is less than 1:5,000 ( $P = <0.0002$ ).

*Blood-flow rates in non-gravid uteri of unilateral pregnancies.* (Curve C.) As a control for distance and a similar vascular tree, the data summarized in curve C were obtained by measuring blood-flow rates from the non-pregnant horn of unilateral pregnancies. As may be seen, no appreciable change in blood-flow rate occurred in this organ until after the twenty-fourth day. The probability that the mean for this day and day twenty-eight may be from the same data is less than 1:5,000 ( $P = <0.0002$ ).

The reason for the decrease in blood-flow toward the end of pregnancy, and which parallels a similar decrease in the rate of blood-flow through the gravid uterus at the same time, is not clear. It is possible that the larger volume of blood (presumably having a higher venous pressure) circulating through the gravid uterus serves to retard the entry of the blood from the non-gravid uterus into the inferior vena cava. Systemic changes may also contribute to the effect (see curve B, fig. 1, and Corbit, 1941).

The divergence between curves A and C up to day twenty is highly significant. Analysis shows that P factors for the means on these curves calculated for days twelve, sixteen, and twenty are  $<0.0054$  (1:180);  $<0.0002$  (1:5,000); and  $0.0001$  ( $<1:10,000$ ). Consequently, a significant difference between local circulation rates is demonstrated between the spherically distended conceptus

site and the non-gravid, undistended contralateral uterus. This difference becomes progressively more marked as pregnancy advances, until a change in shape suddenly relieves and restores the local circulation of maternal blood about the conceptus.

*Blood-flow in interconceptus sites.* A few data on this point are included in table 1, for the twenty-second day, at the time of maximum spherical distention of the uterus by the conceptus. Analysis shows that the rate of blood-flow through these vessels is not significantly different from that found in the contralateral, non-pregnant uterus ( $P = 0.4910$  or about 1:2).

*Resorbing fetuses.* Observations on local blood-flow were made on one resorbing fetus on the twenty-fourth day of pregnancy. The flow differed, but just significantly so, from that in the uterus around the living fetuses on this day of pregnancy ( $P = 0.054$  or 1:18). It was equal to that observed in spherical conceptuses on the twentieth day of pregnancy. It is worth noting and it may be significant that resorbing fetuses on the twenty-fourth day frequently have a spheroidal form, as opposed to the cylindrical shape of the viable conceptuses at this time.

**DISCUSSION.** The results of this study demonstrate that the shape and size of the conceptus affect the flow of maternal blood in the uterine wall about the conceptus. The reason for this bears some consideration, since it is a phenomenon which has not been studied extensively. The general mechanism of hollow viscus accommodation is one of fundamental importance in mammalian physiology, applying in modified form to a number of hollow viscera. The accommodation of the conceptus by the uterus offers an unusual opportunity for its study since retention of the uterine contents is of long duration, and changes in shape of a conceptus are predictable and take place over an extended period of time.

The results of this study make it necessary to conclude that the change in shape and size of the spheres on the twelfth, sixteenth, twentieth, and twenty-second day of pregnancy in the rabbit offer increasing resistance to entry of blood from the maternal arterial tree into the vessels within the uterine wall. Just prior to conversion (elongation), this resistance reaches a critical point.

The factor of *tension* in the uterine wall suggests itself for reasons considered elsewhere (Reynolds, 1946) as one of the causes of this resistance (cf. Hess, 1928). The relationships which obtain in hollow elastic spheres may be summarized as follows:

In spheres,

$$T_s = \frac{r^2}{2} \cdot p$$

where  $T_s$  is the tension at any one point of the surface of a sphere;  $r$ , the radius of the sphere;  $p$ , the pressure within the sphere.

In cylinders,

$$T_c = r \cdot p \text{ and}$$

$$T_l = \frac{r}{2} \cdot p$$

where  $T_c$  is circular tension at any point around the cylinder and  $T_l$ , the longitudinal tension on the surface at any point. In order to prove the correctness of this interpretation of curve *A* of figure 1, it will be necessary to correlate simultaneous determinations of blood-flow, intrauterine pressure, and uterine wall-tensions. Such studies are contemplated.

The view that changing patterns of tension offer similar patterns of resistance to the flow of blood in the smaller vessels in the uterine wall may be regarded as tentatively acceptable. In order to understand the morphological basis of this, the arrangement of the blood vessels in the uterus has been studied by dye-injection and injection-corrosion technics. This will be the basis of a separate report. Suffice it to say that, by injecting Prussian blue into the aorta at 110 mm. Hg pressure, injected-uteri have been obtained which confirm the view *a*, that at the time when the local blood-flow is at its lowest rate the smaller uterine vessels are virtually occluded around the larger spherical conceptuses, and *b*, that elongation to the cylindrical shape permits full and even injection of the whole uterus. The facts of curve *A*, figure 1, are explicable, therefore, on the basis of tension within the uterine tissues on the smallest uterine blood vessels.

One further correlation may be made in conclusion. It is that the restoration of circulation as a result of elongation of the conceptus coincides with a phase of rapid daily increment in fetal weight, and without further growth of the uterus. The circulation is maintained therefore in the face of increasing longitudinal stretching of the uterus. An end to this will come, however, as the limit, or some other critical point, in elongation approaches, near term.

The period when spherical conceptuses are present is also the period of uterine hypertrophy. It would seem to be significant in this connection that tension, which is one of the effective stimuli for uterine growth (Reynolds and Kaminester, 1936) exists to a maximum degree at the time in gestation when uterine growth occurs. Thus the stages of uterine development are seen to be nicely integrated, since in the second trimester of pregnancy, when the fetus is small and blood-flow can be limited, optimal conditions for uterine growth prevail by virtue of the spherical shape of the conceptus. In the last trimester, on the other hand, when circulation must be rapid in order to assure rapid fetal growth and welfare, the stimulus for uterine growth becomes less, and uterine tissue which is made available from uterine hypertrophy in the previous trimester is "paid out" during the elongation process with which the last trimester of pregnancy is associated.

#### CONCLUSIONS

1. Comparison of the blood-flow rate  $\left( \frac{1}{(\text{circulation time})} \right)$  of sodium cyanide injected in small volumes into selected points of the venous system show that:
  - a*. There is a decrease in somatic circulation-rate during the first half of pregnancy (femoral-carotid body) followed by a transient increase to a pre-pregnancy level on the twenty-second day (gestation in the rabbit, 32 days).
  - b*. The circulation-rate of blood in the gravid uterus about the growing conceptus shows three essential features:

(1) There is a progressive decrease in circulation rate until the twentieth day to 66 per cent of the pre-pregnancy level.

(2) Upon attainment of maximum spherical size, about the twenty-second day, a profound decrease in circulation rate is found (to 25 per cent of the pre-pregnancy level).

(3) The conceptus elongates at this point, and there is a restoration of a relatively high circulation rate (to 75 per cent of the pre-pregnancy level) equal to that found upon the twelfth day of pregnancy when the fetus is less than  $\frac{1}{10}$  of its birth weight and about  $\frac{1}{6}$  of its weight on the twenty-fourth day.

c. The circulation rate in non-gravid cornua of unilateral pregnancies shows no change until after the twenty-fourth day, when both local and systemic conditions differ from the pre-pregnancy situation.

d. The circulation rate at interconceptus sites of gravid uteri is the same during the second trimester of pregnancy (until the twenty-second day) as it is in the non-gravid undistended uterus.

e. Within a single uterine cornua at the time of conversion from sphere to cylinder it is found that local blood-flow in the uterus about the elongated (cylindrical) conceptuses is fast, while blood-flow about spherical conceptuses is very slow.

2. Because of the facts noted above, it is necessary to conclude that the local circulation of maternal blood within the uterus varies in an orderly and progressive way, diminishing as the radius of spherically-shaped conceptuses increases, and increasing again (at least at first) as the conceptus becomes cylindrical in the last trimester of pregnancy.

3. The physical explanation of the foregoing conclusion is discussed, and it is tentatively concluded that the physical factor governing the local flow of maternal blood within the uterine wall is local tension within the uterine tissues, the pattern of which is shown to vary during pregnancy with the shape of the conceptus in a way that parallels the blood-flow rates measured in this study.

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# LONG TERM ACCLIMATIZATION TO HEAT

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Alterations in physiological response to heat induced in the human body by continued exposure to conditions of high temperature or to the combination of high temperature and high humidity have been investigated by many physiologists (1-10). The data available provide descriptions of the changes in skin and rectal temperature, pulse rate, sweat loss (7, 8, 9) and sweat composition (10), blood pressure (7, 9), and metabolism (4). These changes, occurring during relatively short term exposures to heat, establish the existence of a physiological adjustment which may be termed acclimatization. The data also indicate that, during this process of acclimatization, the comfort and efficiency of an individual increase (7, 8, 9). The major portion of this adjustment has been observed to occur within the first four or five days of exposure. However, physiological changes occurring during an extended exposure period have never been reported.

In the course of United States Army Quartermaster Corps investigations concerned with evaluation of the physiological heat load imposed by various uniforms, it has been possible to observe the reactions of seven healthy young men performing standard exercise under conditions of high temperature and high humidity for a period of twenty-five weeks. Data presented in this communication reveal the reactions during this long term exposure to these ambient conditions.

**METHODS.** All experiments were conducted between November first and April twenty-fourth in the Jungle Chamber of the Climatic Research Laboratory, Lawrence, Mass. Throughout the study, the temperature of the room was controlled at 90°F., dry bulb, and 86°F., wet bulb (relative humidity) 85 per cent). Temperatures of walls, ceiling, and floor were maintained at the existing dry bulb temperature by circulation of hot water through radiant panels. Daily and day-to-day variations rarely exceeded 1°F., dry bulb, and 0.75°F., wet bulb. Variations in relative humidity were less than  $\pm 2$  per cent. The wind velocity in all studies was 1.5 mph.

Gross physical characteristics of the subjects (seven young, healthy, white soldiers) are presented in table 1. At the initiation of the study, the subjects were physically conditioned by two weeks of field exercise. Thereafter, they were exposed to the designated ambient conditions during a period of one month, each subject spending at least three hours a day, five days a week, in the Jungle Chamber. Of the three hours, at least one was spent in exercise.

<sup>1</sup> Reprints of this paper may be obtained from the Research and Development Branch, Office of The Quartermaster General, Washington 25, D. C.

Collection of the data presented in this report was initiated at the conclusion of this preliminary one-month exposure period. They comprise the results of thirty control experiments conducted during an investigation of the comparative physiological heat loads imposed by uniforms. Throughout the investigation, a control experiment was usually conducted at least once a week, and sometimes more often. These experiments followed a rigidly standardized routine which may be briefly described as follows:

1. After entering the chamber, each subject spent the first hour at rest.
2. Upon completion of this initial hour of adjustment, the subject was weighed with an accuracy of  $\pm 5$  grams, his rectal temperature was determined within the nearest  $0.1^{\circ}\text{F}$ . by means of a clinical thermometer, and his pulse measured by auscultation. Immediately thereafter, the exercise started. Each subject walked for one hour on a horizontal treadmill at the standard rate of 3.5 mph. No rests were permitted and no measurements were recorded during this period. Drinking water was prohibited.

TABLE 1  
*Physical characteristics of subjects*

SUBJECT	HEIGHT	WEIGHT	AREA	HABITUS
	<i>inches</i>	<i>pounds</i>	<i>M.<sup>2</sup></i>	
R. B.....	68	138	1.75	Asthenic
J. E.....	70.5	167	1.95	Asthenic
A. H.....	70.5	167	1.95	Sthenic
J. P.....	73	161	1.98	Asthenic
C. T.....	71	180	2.03	Sthenic
A. W.....	69	185	2.02	Sthenic
J. Z.....	68.5	164	1.89	Sthenic

3. As soon as the subject stepped off the treadmill, his pulse (taken for 30 seconds and multiplied by 2) and rectal temperature were determined. Upon completion of these measurements, he was wiped dry of all perspiration and weighed.

4. After weighing, the activity of the subject was unrestricted, but he was required to remain in the experimental chamber for an additional hour.

Two subjects walked on the treadmill simultaneously. All wore similar clothing, namely, cotton shorts, light wool socks, and jungle boots. This gear was standard throughout the exposure period. No attempt was made to have the subjects on the treadmill at the same time each day. Precautions were taken to insure that a high level of health was maintained. Weights were recorded daily and urine chlorides checked periodically to be certain that no dehydration or salt deficit developed.

RESULTS. The data obtained illustrate the physiological response of seven healthy males to prolonged exposure in ambient conditions of high temperature and humidity. Mean values and standard deviations for the rectal temperatures and pulse rates of each subject are presented in table 2. It will be seen that

these values were reproducible (within the limits described) from day to day over a long period and that no definite trend occurred. However, during this

TABLE 2  
*Summary of data on rectal temperature and pulse rate*

SUBJECT	RECTAL TEMPERATURE		PULSE RATE	
	Mean	Standard deviation	Mean	Standard deviation
	$^{\circ}\text{F.}$		<i>per minute</i>	
R. B.....	100.64	0.32	126.8	10.3
I. E.....	100.56	0.24	130.5	10.1
A. H.....	99.87	0.23	124.8	10.0
J. P.....	100.91	0.31	109.2	9.5
C. T.....	100.15	0.23	108.2	9.1
A. W.....	100.23	0.22	107.8	8.2
J. Z.....	100.66	0.30	129.3	11.1

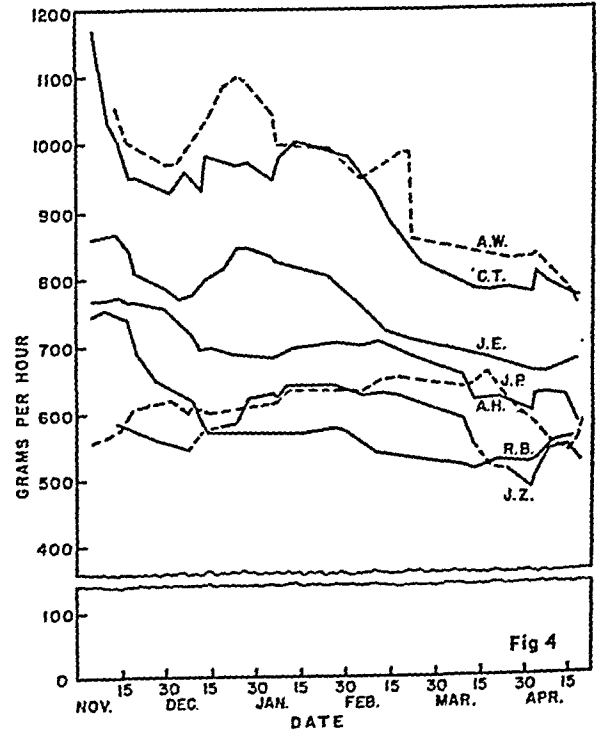
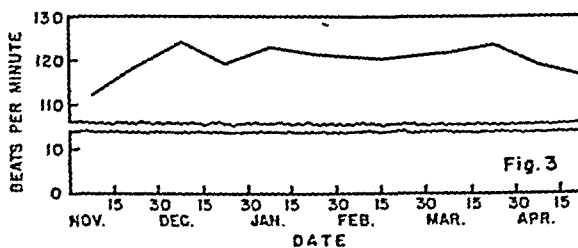
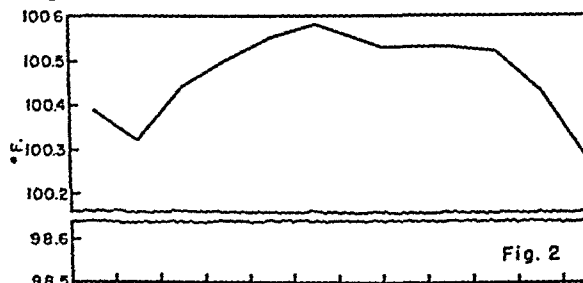


Fig. 1. Mean weight loss—5 exposure moving average.

Fig. 2. Mean rectal temperature—5 exposure moving average.

Fig. 3. Mean pulse rate—5 exposure moving average.

Fig. 4. Individual values for weight loss—55 exposure moving average (values in grams).

six-month period, there appeared to be a slight but definitely downward trend in weight loss.<sup>2</sup> This trend is demonstrated by a comparison of figures 1, 2, and 3,

<sup>2</sup> Weight loss and sweat loss are used interchangeably. Decrease in weight due to loss of water through respiratory tract and loss of carbon dioxide is considered constant and negligible.

illustrating, respectively, the average weight loss, rectal temperature, and pulse rate. In order to smooth the curves so that the results may be easily interpreted, each point in these graphs represents a five-exposure moving average.

The results presented in figure 1 reveal a decline in the mean value for weight loss. At the initiation of the experimental period, the mean weight loss was 825 grams per hour. At the conclusion, this value had declined to 640 grams per hour. A detailed presentation of these data is provided in figure 4. In this figure, the five-exposure moving average has been calculated for each of the seven subjects. Five exhibit a definite decline in sweat production. Two, J. P. and J. Z., show no constant deviation throughout the period. Nothing unusual was observed relating to the body habitus, previous experience, or ease of work performance which distinguished these two groups. However, the weight losses of J. P. and J. Z. were initially lower than the comparative values of other subjects similar in size and body build.

Five-exposure moving averages for rectal temperatures and pulse rates (figs. 2 and 3) indicate no significant variation in either direction. The dissociation between weight loss and these values is striking. Although the weight loss declined, the rectal temperature and pulse rates remained unaltered.

DISCUSSION. Previous studies of acclimatization emphasize the salient changes in physiological adjustment during the first four or five days of exposure to hot environment. Numerous investigators have stated that, at the conclusion of such a five-day period, a subject is able to complete a given work period with lower rectal temperature, pulse rate, skin temperature, and more stable blood pressure (7, 8, 9). None of these investigators, however, has reported data extending over a period as long as the term of the experiment described in this communication. Since no striking improvement had appeared after the fifth day, it probably had been assumed that acclimatization was complete, that a stable state had been attained.

It could be presumed, therefore, that a similar condition existed at the initiation of this experiment, the subjects having been exposed to a hot environment for one month before collection of data. During the experiment, exposures were made almost daily for a period of 6 months and physiological data were periodically collected. The results demonstrate that in a majority of subjects, except for minor fluctuations, there was an over-all decline in sweat rate during continued exposure to the ambient conditions of this experiment. This phenomenon was more marked in the two subjects with high initial sweat rates. Two subjects with low initial sweat rates showed no change. The range between the individual rates was greater at the beginning of the study than at the end, i.e., there was a tendency to approach a common basal value for sweat production. Even at the termination of the six-month period there was no evidence that the progressive decline had ceased. Thus, in a majority of subjects, acclimatization, as evidenced by decrease in sweat rates, is a continuous process.

It must also be noted that no downward trend in the rectal temperature and pulse rate accompanied the sweat rate. While the data demonstrate that the adjustment to high temperature and high humidity continues over an



extended period, they do not indicate that there was a significant increase in working ability. The subjects were more efficient and their adjustment more satisfactory only in that they did not produce as much sweat. However, under the conditions of this experiment an excess of perspiration is produced. The reduction, therefore, is only of importance because it effects a slight economy in the salt and water requirements of the subjects.

The data also indicate that there is no constant relationship between rectal temperature and sweat rate—the latter values dropped while the former remained constant. The progressive decline in sweat rate may account for the observation that persons who have undergone prolonged exposure to high temperature do not produce as much sweat as those recently exposed (11).

#### SUMMARY

This report presents data relating to the physiological response of seven healthy males to daily exposure in conditions of high temperature and humidity during an extended period. The subjects, after being acclimatized for one month, were exposed to these ambient conditions for six months. The results indicate that there is no trend in either the pulse rates or rectal temperatures. However, five of the seven subjects exhibited a decline in sweat rate throughout the experimental period.

The implications of these observations are briefly considered.

The author wishes to acknowledge the assistance of Miss Agnes M. Galligan and Mrs. Marie A. Fitzgerald.

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# THE INTESTINAL SYNTHESIS OF NIACIN AND FOLIC ACID IN THE RAT<sup>1</sup>

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That vitamins are synthesized by intestinal microorganisms has been proved beyond any reasonable doubt. In some cases, significant amounts of these vitamins are absorbed from the intestines and utilized in the animal body. The evidence is particularly strong in the case of folic acid. Intestinal microorganisms have been shown to synthesize folic acid *in vitro* (1, 2) and the vitamin has been found in a simple water extract of the cecal contents as well as in the washed intestinal walls (3). Rats on certain highly purified diets containing very small amounts of folic acid grow well, but when sulfa drugs are added, poor growth and granulocytopenia are observed (4, 5), concurrent with marked changes in the intestinal flora (6, 7). When folic acid concentrates or pure folic acid compounds are administered, growth is resumed and the blood picture improves (8, 9, 10). The addition of sulfa drugs to synthetic diets definitely reduces the vitamin B<sub>12</sub> potency of rat livers; when sources of vitamin B<sub>12</sub> are added, the vitamin B<sub>12</sub> potency of the livers increases several fold (11, 12).

Similar evidence does not exist in the case of niacin, though it is clear that this vitamin is synthesized somewhere in the rat's body (13, 14). Dann (14) concluded that intestinal synthesis is not an important source of niacin for the rat because its addition to purified diets containing 1 per cent sulfaguanidine did not produce increased growth. However, a response would not be expected because folic acid was not supplied in his ration. Furthermore, it is well known that sulfa drugs may change the species comprising the intestinal flora without changing the total number of organisms, and in the light of present knowledge, the amount and type of drug fed in the experiments of Dann would not be expected to produce a maximum effect.

Thus the question of whether or not sufficient synthesis of niacin for the rat's needs takes place in its tissues remained unanswered and the present studies were designed to throw some light on the matter. Data on the intestinal synthesis of niacin on various rations were needed as a basis for the study of the pellagra-preventing action of such foods as milk which is low in niacin and the pellagra-promoting action of corn. Folic acid determinations were made as an adjunct to the main study.

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The choice of a method for studying intestinal synthesis is dictated by available facilities and the type of information desired. The use of sterile animals has been suggested as an ideal approach but this involves a great deal of expense and effort, and while it provides valuable information, it does not offer final proof of what happens under normal conditions. The excretion of nutrients may be measured and compared with the intake (16). In addition the average amount of the vitamin found in the entire animal at the beginning and end of the experiment may be determined (14, 17). Additional techniques include the use of drugs, cecectomy, dietary modification and careful studies of the intestinal flora. None of these methods obviates the possibility of synthesis in the tissues and subsequent excretion into the intestinal tract.

Under most conditions the cecum contains the greatest part of the intestinal contents and a number of studies point to this structure as an important seat of vitamin synthesis (18, 19). Taylor, Pennington, and Thacker (20) found that cecectomy of rats on a poor diet had a deleterious effect on growth while cecectomy on a good diet had no effect. Wakim, Krider and Day (20) have reported that cecectomy produces more consistent hypoprothrombinemia in rats on purified rations containing sulfa drugs. Kornberg, Daft and Sebrell (21) tested the cecal contents of rats on diets with and without sulfa drugs in the diet and found that the latter had greater activity in counteracting prothrombin deficiency in rats.

In the present studies the needed information was obtained through the determination of niacin and folic acid in the cecal contents of rats on various diets after they had been on experiment for 1 and 2 weeks. In addition, a highly purified diet complete in all known factors for the rat except niacin was fed to rats together with sulfa drugs in an attempt to produce a niacin deficiency.

**EXPERIMENTAL.** Twenty-one day old male weanling Sprague-Dawley rats were placed on the different rations and at the end of 1 and 2 weeks the cecal contents of 3 rats in each group were analyzed for niacin and folic acid. The basal diet had the following composition:

Carbohydrate.....	78 grams
Casein.....	15 grams
Corn oil.....	3 grams
Salts IV <sup>2</sup> .....	4 grams
Cystine.....	0.15 gram
Thiamine.....	0.2 mgm.
Riboflavin.....	0.3 mgm.
Pyridoxine.....	0.25 mgm.
Ca-pantothenate.....	2.0 mgm.
Biotin.....	0.005 mgm.
2-methyl-1,4-naphthoquinone.....	0.1 mgm.
Choline.....	100 mgm.
Inositol.....	10 mgm.
Folic acid preparation $\cong$ 1 per cent solubilized liver extract powder containing	
11.5 ug. B <sub>12</sub> ( <i>S. faecalis</i> assay) per 100 grams ration.	
$\alpha$ -tocopherol.....	1 mgm. per week
3 drops Haliver Oil diluted 1-6 with corn oil per week.	

<sup>2</sup> Phillips, P. H. and E. B. Hart. J. Biol. Chem. 109:657, 1935.

The folic acid preparation contained only 1.6 ug. of niacin per 11.5 ug. B<sub>6</sub> (22). The basal diet without the folic acid preparations contained less than 0.01 mgm. niacin per 100 grams.

Phthalylsulfathiazole was added at the expense of the entire ration. The lactose and skim milk powder were added to the synthetic rations at the expense of sucrose. Mineralized skim milk powder was prepared so that each 100 grams of ration contained the following amounts of added minerals: 15 mgm. iron, 15 mgm. copper and 1.5 mgm. manganese. Vitamins A, D and E were supplemented by dropper as on the basal ration.

"Synthetic milk" had the following composition:

Carbohydrate (lactose unless specified) . . . . .	40
Casein . . . . .	28
Fat . . . . .	26
Salts IV . . . . .	6
Vitamins as in basal diet above	

In all cases the casein (Labco) was extracted three times with hot 95 per cent ethanol.

The animals were killed by decapitation and the cecal contents were suspended in 0.1 N KOH and autoclaved at 121°C. for 1 hour. The suspensions were neutralized with HCl and stored in the cold under chloroform and toluene.

Niacin was determined with *Lactobacillus arabinosus* according to the method of Krehl, Elvehjem and Strong (23). The folic acid potency was measured by the method of Teply and Elvehjem (24) using *Streptococcus faecalis* with vitamin B<sub>6</sub> as the standard. In preliminary studies the vitamin B<sub>6</sub> potency of cecal material was not materially increased by various treatments with enzymes, acid or alkali. However, since autoclaving for 1 hour with 0.1 N KOH did not destroy the activity of vitamin B<sub>6</sub> for either *S. faecalis* or *Lactobacillus casei* this treatment was used as standard procedure. P-aminobenzoic acid was added to the tubes to counteract any sulfa drug present in the cecal contents.

In all cases the cecal contents represented the greatest part of the intestinal contents. On lactose diets and on some of the diets containing sulfa drugs the ceca were greatly enlarged. There was a variation in color and consistency ranging from watery white material on the lactose diets to black pithy material on the dextrin diet.

The figures given in table 1 are averages of three analyses. In most cases there was remarkably close agreement within each group.

The largest amount of niacin and folic acid per gram of cecal contents was found in the rats on the dextrin ration. On a high lactose diet there was little synthesis of either vitamin during the second week. This was probably due to an excessive amount of unabsorbed lactose in the cecum. The addition of sulfa drug to the lactose ration actually caused an increased amount of niacin in the cecum. This effect also occurred in the case of some of the milk powder diets.

In general the inclusion of 2 per cent phthalylsulfathiazole caused a marked decrease in cecal niacin on a per gram basis, but the ceca increased in size so

that there was practically no change in the total amount present. In contrast, the folic acid content was greatly decreased even on a "total amount" basis.

TABLE 1

*Niacin and folic acid in rat cecal contents (averages of values from three different rats)*

DIET	NIACIN		WT. OF CECAL CONTENT		FOLIC ACID**	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
	ug. per gm.	ug. per gm.	gm.	gm.	ug. per gm.	ug. per gm.
Sucrose basal.....	19.3	24.7	0.28	0.40	0.53	0.85
Sucrose basal + 2% phthalylsulfathiazole.....	2.8	3.5	1.9	3.9	0.02	0.03
Lactose basal.....	42.6	0.9	0.33	3.5	1.8	0.03
Lactose basal + 2% phthalylsulfathiazole.....	4.3	8.8	2.3	5.5	0.03	0.01
Glucose basal.....	18.6	13.3	0.49	0.67	0.6	0.3
Glucose basal + 2% phthalylsulfathiazole.....	3.0	4.8	2.3	2.7	0.02	0.02
Dextrin basal.....	31.1	48.7	0.66	1.0	1.9	1.0
Dextrin basal + 2% phthalylsulfathiazole.....	2.6	3.3	2.1	5.3	0.01	0.006
Sucrose basal + "1% folic acid"*.....	30.1	31.5	0.56	0.82	1.3	0.4
Sucrose basal + "1% folic acid"* + 2% phthalylsulfathiazole.....	2.3	5.3	3.5	3.8	0.2	0.04
Sucrose basal + 0.5 mgm.% niacin.....	33.9	24.0	0.40	0.69	1.0	
Sucrose basal + 0.5 mgm.% niacin + 2% phthalylsulfathiazole.....	3.2	3.6	1.7	3.1	0.07	
Sucrose basal + 15% skim milk powder.....	42.1	35.6	0.83	1.2	1.7	0.3
Sucrose basal + 15% skim milk powder + 2% phthalylsulfathiazole.....	3.9	9.9	1.4	2.3	0.01	
Sucrose basal + 7½% lactose.....	31.9	30.9	0.41	0.55	0.9	
Sucrose basal + 7½% lactose + 2% phthalylsulfathiazole.....	2.9	4.9	2.2	3.4	0.03	
Mineralized skim milk powder + 30% corn oil.....	11.9	4.1	2.1	3.9	0.03	
Mineralized skim milk powder + 30% corn oil + 2% phthalylsulfathiazole.....	5.4	15.9	2.2	4.0	0.02	
Mineralized skim milk powder + 30% butterfat.....	6.9	15.8	1.5	3.8	0.1	
Mineralized skim milk powder + 30% butterfat + 2% phthalylsulfathiazole.....	15.6	16.4	1.7	3.4	0.04	
"Synthetic milk" (corn oil).....	12.5	17.0	0.85	1.9	0.1	
"Synthetic milk" (butterfat).....	20.8	36.6	0.82	1.1	0.4	
"Synthetic milk" (butterfat-sucrose).....	15.3	16.1	0.46	0.60	1.0	

\* Special "folic acid" concentrate = 1% solubilized liver extract or 11.5 ug. B<sub>9</sub> per 100 grams of ration.

\*\* Potency compared with vitamin B<sub>9</sub> using *S. faecalis*.

This may explain the relative difficulty of producing niacin deficiency with sulfa drugs particularly when adequate amounts of folic acid are present.

On the "synthetic milk" ration only moderate amounts of folic acid were found. Replacement of lactose with sucrose increased the folic acid activity of the cecal

contents and decreased the niacin content. Substitution of butterfat for corn oil allowed an increased synthesis of both vitamins.

On mineralized milk powder +30 per cent corn oil or butter fat only moderate amounts of folic acid were found in the cecum. Phthalysulfathiazole diminished the amount slightly. The amount of niacin in the cecum of rats fed the corn oil ration was low the first week and increased during the second week. When butterfat was used instead of corn oil the niacin content in the cecum was high

TABLE 2

*Growth depression with phthalysulfathiazole and its counteraction with niacin (weekly average gains of three rats in each group)*

DIET USED	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	AVERAGE
	gm.	gm.	gm.	gm.	gm.
Basal diet.....	32	32	30	31	31
Basal diet + 2% phthalysulfathiazole.....	19	15	8	5	12
Basal diet + 2% phthalysulfathiazole + "1% folic acid"*.....	14	15	17	17	16
Basal diet + 2% phthalysulfathiazole + 0.5 mgm.% niacin.....	19	9	4	7	13
Basal diet + 2% phthalysulfathiazole + "1% folic acid" + 0.5 mgm.% niacin.....	23	27	29	31	28

\* As in table 1.

TABLE 3

*Growth depression with high levels of phthalysulfathiazole and its partial counteraction with niacin (average weekly gains of three rats in each group)*

DIET USED	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
	gm.	gm.	gm.	gm.
Basal diet.....	24	36	32	37
Basal + 4% phthalysulfathiazole.....	12	11	9	1
Basal + 4% phthalysulfathiazole + "1% folic acid"*.....	10	16	27	22
Basal + 4% phthalysulfathiazole + "1% folic acid" + 1 mgm. % niacin.....	18	21	26	20

\* As in table 1.

the first week and remained at a high level. It is interesting that only in the case of milk powder diets was there no change in the size of the cecum on the addition of phthalysulfathiazole.

The addition of 7½ per cent lactose or 15 per cent skim milk powder to the sucrose basal ration caused a marked increase in niacin and folic acid in the ceca. The addition of phthalysulfathiazole reduced the amounts to those obtained with the basal diet plus phthalysulfathiazole. Although milk may provide "bound folic acid" which can be used by the rat (25) it probably contributes to the synthesis of this vitamin because of its effects on the intestinal flora.

Addition of excess niacin increased the synthesis of folic acid and excess folic acid increased niacin synthesis. These effects were eliminated by feeding phthalylsulfathiazole.

Although the present studies are primarily concerned with synthesis of vitamins, the concurrent destruction of niacin probably plays an important rôle in determining the final niacin concentration (26).

*Attempts to produce niacin deficiency.* The basal diet described above was employed. In preliminary experiments succinylsulfathiazole at a 1 per cent or 2 per cent level produced severe diarrhea. Phthalylsulfathiazole caused much less diarrhea and was used exclusively in the experiments described.

Table 2 shows that with a diet containing 20 per cent casein and 73 per cent sucrose the addition of 2 per cent phthalylsulfathiazole produced a growth depression that could not be counteracted by an amount of folic acid concentrate  $\cong$  1 per cent solubilized extract powder. Supplementation with both folic acid and 0.5 mgm. per cent niacin almost completely counteracted the effect of phthalylsulfathiazole.

The results in table 3 were obtained in an experiment where the level of the drug was raised to 4 per cent. After the first two weeks niacin did not potentiate the action of folic acid. At the 4 per cent level of phthalylsulfathiazole niacin and folic acid together could not counteract the effect of the drug. Whether the limitation is related to one or both of these two vitamins or due to some other effect is not known. Neither substituting glucose for sucrose nor reducing the casein content of the diet to 15 per cent has made the niacin deficiency more marked.

The increased effectiveness of folic acid after the second week on experiment can probably be attributed to the increased intestinal synthesis of niacin as a result of cecal growth. Taylor et al. (20) state "At the time of weaning, the rat's cecum is only about one-fourth as large relative to the digestive tract as it will be in the adult. This may account in part for the well-recognized fact that rats at this stage are less resistant to some vitamin deficiencies than they are a week or so later."

As far as the authors are aware these experiments are the first to indicate that a niacin deficiency may be produced in the rat through the use of sulfa drugs. It has been shown that the ration must be highly purified and high levels of a suitable sulfa drug must be used if positive results are to be obtained. It is hoped that the availability of pure folic acid and new drugs will facilitate further studies.

#### SUMMARY

1. The ceca of rats that had been on various diets with and without 2 per cent phthalylsulfathiazole for 1 and 2 weeks were analyzed for niacin and folic acid.
2. Dextrin caused the greatest synthesis of both niacin and folic acid.
3. In general, 2 per cent phthalylsulfathiazole produced a marked decrease in the concentration of niacin in the cecal contents, but the ceca increased in size so that the total amount remained about the same. The folic acid content was greatly decreased even on a total basis.

4. Excess niacin increased the synthesis of folic acid and excess folic acid increased the synthesis of niacin. These effects were prevented by feeding 2 per cent phthalylsulfathiazole.

5. Addition of lactose or milk powder to the basal ration increased the synthesis of both niacin and folic acid. When phthalylsulfathiazole was fed this increase did not occur.

6. On "synthetic milk" diets butterfat caused more vitamin synthesis than did corn oil.

7. Through the use of a highly purified diet growth depression was produced with phthalylsulfathiazole which could be counteracted only by feeding niacin along with folic acid.

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# EXPERIMENTAL TRAUMATIC SHOCK PRODUCED BY MUSCLE CONTUSION WITH A NOTE ON THE EFFECTS OF BULLET WOUNDS. A STUDY OF THE CLINICAL<sup>1</sup> SIGNS OF SHOCK IN THE DOG AND OF THE RÔLE OF BLOOD VOLUME REDUCTION IN THE DEVELOPMENT OF THE SHOCK SYNDROME<sup>2, 3</sup>

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The experiments described here, carried out in the fall-winter of 1941, constitute the first of a series of investigations of shock undertaken by this laboratory during the war years. The work was started with the very definite aim of orienting such physiological studies as we might make around the clinical picture, that is, to use the signs and symptoms of shock as the frame of reference. It was apparent that in many investigations of experimental shock the attempt had been to reproduce as faithfully as possible certain types of injuries which were known to cause shock in man. However, the truth must be stated that in most of these experiments, the evidence as to whether or not the animals actually succumbed in a state of shock was far from convincing and indeed this evidence could not have been obtained because the experiments were of such character that they had to be done under a general anesthetic. It was our conviction that a prerequisite for further investigation of shock was to determine, on the basis of signs and symptoms, whether or not the condition produced experimentally was a true replica of the syndrome of shock as observed in man. Only then could one be reasonably certain that the experimental findings were true manifestations of shock and not the result of irrelevant experimental factors such as anesthesia. As will be seen, the required conditions were in part realized by adopting an experimental procedure in which the use of general anesthesia<sup>1</sup> could be discontinued after the injury had been inflicted, thus permitting the subsequent development of the clinical signs. Even so, we had serious reservations concerning the possible distortions arising from the use of ether anesthesia while the animal was being traumatized. We now have evidence at least in relation to the resistance to lowered blood volume that this factor does not play a significant rôle in the eventual response to the trauma.

Another immediate objective of these experiments was to determine the degree of blood volume reduction in traumatic shock and its rôle in the development of

<sup>1</sup> The word "clinical" is employed in this paper in the same sense that it is used in the hospital or clinic.

<sup>2</sup> This work was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University.

<sup>3</sup> The blue dye, T-1824, was furnished by the Warner Institute for Therapeutic Research, New York City.

the shock syndrome. As may be seen from the literature preceding 1941, current opinion on this point presented a curious state of affairs. On the one hand it was firmly believed and widely stated that the loss of circulating blood volume was one of the central features of shock. On the other hand, the direct evidence from blood volume determinations which certainly supported this belief, was not accepted as valid, at least quantitatively. In the first place, there had been, and still is, some controversy over whether or not any of the available blood volume techniques actually measured the true blood volume. Secondly, the obvious disturbance in blood flow in deep shock, and the widely accepted notion that excessive capillary leakage occurred in shock were considered adequate grounds for suspecting that the blood volume could not be determined by any of the dilution methods. Hence, the central problem was really to ascertain whether or not the methods were applicable in shock. Since we first collected the results included in the present paper, we have investigated the technical problem further (36, 47) and on the basis of these subsequent findings now believe that our original data presented here are essentially correct. We have also had occasion in the meantime to extend the investigations to a large series of clinical cases of shock (17, 42, 48), which substantially confirm the results obtained in the experiments on dogs.

The present report consists of three sections. The first deals with the clinical signs and symptoms of shock in the dog. The second is a brief note on the production of shock by bullet wounds and the third is concerned with the changes in blood volume caused by muscle contusion.

A. SHOCK SYNDROME IN THE DOG. *Methods.* The experiments considered in this section were done on 30 mongrel dogs, 15 males and 15 females, ranging in weight from 7 to 15 kgm. During the control and post-traumatic periods the dog was placed on its back on a trough-shaped animal board designed to support the animal comfortably in a supine position. With gentle handling and careful attention to the animal's comfort, signs of fear and excitement soon disappeared even in untrained dogs. During a one hour control period, the rectal temperature was measured, the heart rate was counted repeatedly by means of a stethoscope and the mean arterial pressure was determined by femoral arterial puncture. At the same time special attention was given to other signs of clinical significance, such as evidence of the animal's interest in his surroundings and his responsiveness to petting, the relative temperature of the extremities, the appearance and texture of the oral mucous membranes, the size of the jugular veins and the color of the jugular venous blood.

Ether anesthesia was induced by the drop method and the hair removed from both hind legs with clippers. Then, with the animal under full surgical anesthesia, the thigh muscles were contused with a light raw-hide mallet (weight 182 grams, diameter of striking surface, 4 cm., length of handle, 20 cm.). The sequence of events described below was usually produced by 700 to 1000 blows on each leg. The number of blows required varied, as one might expect with the force of each blow, with the size of the animal and the susceptibility of its skin and muscles to bruising. During this and the subsequent investigations we

sought constantly to standardize the procedure, and although we were able to produce fatal shock consistently in a high percentage of the animals we never succeeded in eliminating the element of "feel" or subjective personal judgment from the experiments. As the work progressed it became apparent that the results were most consistent if the injury was applied by the same individual from day to day. The blows were distributed on the inside as well as the outside of the thighs, mostly over the region of the hamstring muscles, care being taken not to strike the same area too many times to avoid perforation of the skin and external loss of blood and tissue fluid. Limiting the blows to the muscular regions of the extremity also avoided injury to bones, large blood vessels and nerves. This procedure is essentially the technique employed by Best and Solandt (1).

As soon as the injury had been inflicted, the administration of ether was discontinued and the clinical condition of the dog was studied until death occurred or it was apparent that the dog was recovering. The heart rate, the rectal temperature, and the blood pressure were determined at regular intervals and notations were made regarding changes in the appearance of the mucous membranes, the relative temperatures of the extremities, the fullness of the jugular veins and the color of the blood drawn from these vessels. Autopsies were carried out on all of the dogs that died.

*Results.* The contusions produced marked swelling of and hemorrhage into the tissues. The swelling extended into the flank and groin. The first few hundred blows usually caused a greater degree of swelling than did the later blows. In a few dogs in which considerable injury had first been inflicted upon one leg, the contusion of the opposite thigh muscles resulted in relatively little swelling. It is probable that the generalized vasoconstriction and fall in blood pressure limited the fluid loss when the second leg was traumatized. Post-mortem inspection of the traumatized tissues revealed that no large vessels had been ruptured. There were superficial and deep areas of macerated tissues but in between the muscle fibres appeared normal. That the tissue damage was by no means complete or severe enough to abolish function of the muscle groups is shown by the fact that on recovery from anesthesia the animals were able to stand and walk about. There were indications of a certain amount of proprioceptor deficiency for the feet were dragged slightly when the dogs walked.

*Mortality and survival times.* This first series of experiments gives a fair impression of the extent to which we were able to reproduce the same state or severity of shock by muscle contusion from day to day. According to the clinical signs described below, 29 of the 30 dogs developed shock and of these 25 succumbed in shock. The survival times of the latter ranged from 50 minutes to 9 hours and 21 minutes. However in 21 of the dogs the survival time fell between 2 and 5½ hours, the average being 3 hours and 20 minutes. Thus it is apparent that although the amount of injury can be gauged so as to cause fatal shock in nearly 85 per cent of the animals, the procedure is not sufficiently refined to produce a narrow range of survival time. It is probable that the variations in response could have been reduced somewhat by utilizing dogs of uniform age, size and breed.

*Pattern of severe shock.* The charts presented in figures 1 to 6 illustrate the general effects of the trauma on the heart rate, blood pressure and body temperature as observed in dogs that succumbed to the injury. In dog 18 for example (fig. 1), the heart rate immediately after trauma is more than double the control value (from 81 to 168 beats per minute). After this the heart rate continues to rise slowly over a period of  $3\frac{1}{2}$  hours to a maximum of 222 beats per minute. The record then shows the beginning of a sharp downward trend, which in our experience invariably appeared shortly before death (see also fig. 3). The mean arterial pressure which was 100 mm. Hg during the control period dropped to 73 mm. Hg after the trauma had been inflicted. This level was maintained for

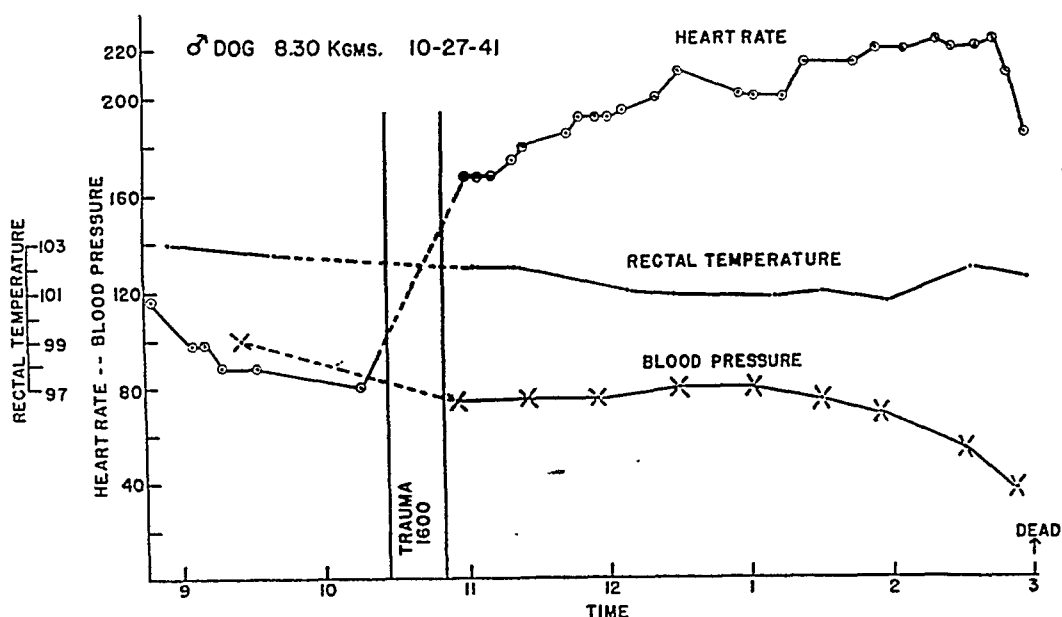


Fig. 1. The effect of muscle trauma upon the heart rate, blood pressure and rectal temperature of dog 18. After injury the limbs became progressively colder, and the oral mucous membranes became more pale. The jugular veins were so constricted that the dark blood present in these vessels was drawn with difficulty. During the last hour of the experiment respiration was labored and the animal showed considerable central nervous depression. Immediately before death the dog began to gasp and the forelegs were rigidly extended.

two and a half hours after which time the pressure declined gradually. The post-traumatic rectal temperature was about  $2^{\circ}\text{F}$  below the control value. After injury the dog's limbs became progressively colder, and the oral mucous membranes increasingly pale and dry. The jugular vein blood became strikingly darker in color and the vessels so constricted that samples could be drawn only with considerable difficulty. Three hours and 40 minutes after the injury, respiration was noticeably deeper and soon thereafter it became labored. The animal also showed considerable nervous depression. He lay quietly, taking little or no interest in his environment. The pupillary reflexes became more and more sluggish. About 4 hours after injury the dog began to gasp, the forelegs

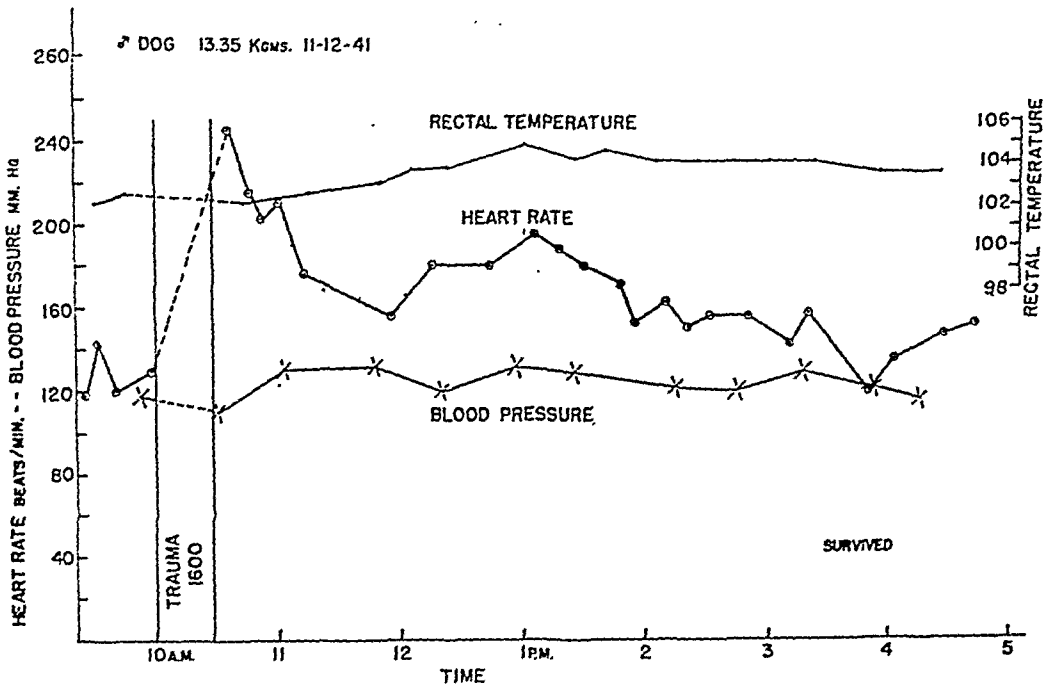


Fig. 2. The effect of muscle trauma on dog 26. Shock was not produced. Note the high post-traumatic blood pressure. The heart rate fell from 240 beats per minute immediately after injury to 150 beats per minute over a period of several hours. Although the oral mucous membranes were cold, the dog showed no central nervous depression. The rectal temperature increased.

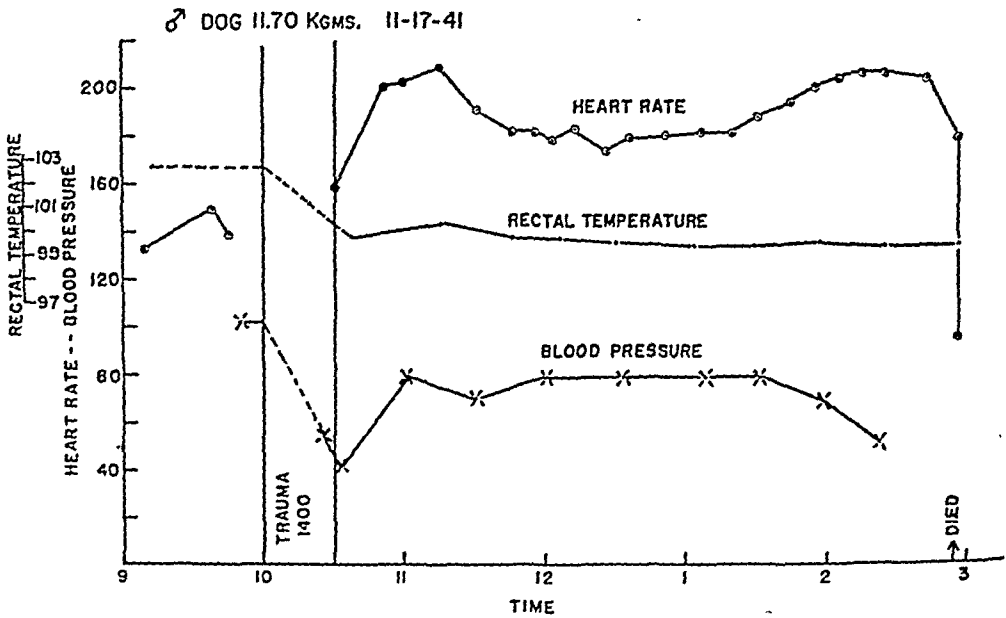


Fig. 3. The effect of muscle trauma on dog 5. Thirteen hundred blows decreased the mean blood pressure from a control level of 103 to 55 mm Hg. An additional 100 blows further reduced the pressure to 30 mm. Hg. The terminal bradycardia shown in this figure was a conspicuous feature in dogs which died after muscle injury.

were rigidly extended, respiration ceased, and the heart beat was no longer perceptible.

*Responses to injury without shock.* Only one of the 30 dogs failed to show the characteristic signs of being in shock. The clinical chart on this dog is presented in figure 2. It will be seen that the blood pressure during the entire period of observation following the trauma is above the control level, and the heart rate, although high (240 per min.) immediately after the injury, shows a general downward trend instead of a rise during the succeeding hours. Furthermore, the rectal temperature goes up (from 102 to 104°F.) instead of down. Although the oral mucous membranes were pale and the extremities seemed somewhat colder than before injury there were no evidences of central nervous depression, or noticeable changes in the respiration. The blood samples obtained from the jugular veins were normal in color and readily withdrawn. The clinical picture presented by this dog after trauma is clearly quite different in nearly all respects from that described in the preceding paragraph, and yet the amount of injury, in terms of the number of blows applied (1600), is of the order of magnitude which in other animals produced fatal shock.

*Signs of shock.* A brief consideration of our observations concerning each of the signs and symptoms by which the clinician commonly defines shock reveals some important variations in the phenomena associated with shock and their relation to the severity of injury.

*Blood pressure.* The blood pressure was invariably low at the end of the period of trauma. A part of this reduction in pressure is apparently the direct result of afferent stimulation from the blows, for in most animals there was a considerable recovery in blood pressure within a few minutes after the traumatization was stopped (see fig. 3). Furthermore, depressor effects arising from the injured extremities were observed incidentally on several occasions when the region around a nerve trunk (femoral) was subjected to gentle pressure or manipulation. We obtained some evidence that the fall in pressure during the contusion was proportional to the number of blows. This is illustrated in figure 3 on dog 5. Thirteen hundred blows lowered the pressure from the control level of 103 to 55 mm. Hg. An additional 100 blows reduced the pressure to 39 mm. Hg. However, as noted above, the degree of fall during the period of trauma does not apparently bear a constant relation to the post-traumatic pressure level. Also, it is not a consistent index of the severity of the trauma probably because the nervous factor is not constant from experiment to experiment as indeed the later statistical analyses seem to indicate (40, 41).

The course of the blood pressure after trauma was highly variable and unpredictable. However, in the most severely injured animals the pressure was usually low after the contusion and soon took a downward course presaging a short survival time (see dogs 2, 7, 10, 20 and 30 in table 1). In some experiments the low level of pressure was maintained fairly constant for several hours (figs. 1 and 3), whereas in others even where the level was fairly high immediately after trauma the blood pressure declined steadily from the beginning (figs. 4 and 5). In 2 animals (dogs 8 and 27, table 1) the blood pressure was maintained at nearly

normal levels until just before death although by all other criteria the dogs were judged to be in severe shock. It is apparent from these observations that

TABLE 1  
*The effect of muscle trauma upon the blood volume of dogs*

DOG. NO.	WT.	CONTROL				TIME OF INJ.	POST TRAUMA				SUR- VIVED
		Plasma vol.	Blood vol.	Hct.	Pl. Prot.		Plasma vol.	Blood vol.	Hct.	Pl. Prot.	
	kgm.	cc.	cc.	%	gm. %	min.	cc.	cc.	%	gm. %	min.
1	8.4	373	597	39	5.9				43	6.6	168
2	14.4			43	6.0	64	398	695	44.5	5.7	140
3	7.1	396	653	41	4.6				46.5	5.5	560
4	9.8	489	726	30	5.1				34	4.7	334
5	11.7	545	833	36	6.4	134	285	495	44	6.9	238
6	14.6	808	1450	46	6.8	259	498	1015	53	6.7	∞
7	8.5	554	739	26	5.0	40	240?	361	35	5.5	50
8	8.8	548	946	44	5.5	308	295	568	50	6.5	390
9	12.0	703	1282	47	5.4	217	341	668	51	5.2	300
10	9.2	575	893	37	5.7	28	328	525	39	4.9	120
11	8.3	565	1000	45.5	7.3	181	292	584	52	7.8	250
12	8.6	530	967	47	5.2	120	297	551	48	5.2	300
13	10.4	447	708	34	5.4	150	260	403	37	5.2	∞
14	9.3	660	1055	39	7.6	45	392	590	35	6.9	230
15	10.6	635	1097	44	6.8	45	409	726	45.5	6.4	248
16	8.5	587	1060	47	6.4	45	381	747	51	6.6	∞
17	8.1	490	778	38.5	5.2	35	304	454	34.5	4.7	187
18	8.3	600	866	32	5.8	190	310	503	40	5.9	250
19	8.5	515	1028	52	6.7	25	250	480	50	6.8	252
20	15.0	965	1567	40	6.0	88	435	735	42	6.0	123
21	14.4	720	1170	40	6.5	59	412	840	53	6.9	156
22	7.0	375	660	45	5.8	265	188	362	50	6.3	286
23	8.3	469	800	43	6.3	60	325	610	48.5	6.7	∞
						220	312	600	50	6.5	
24	8.8	472	785	41.5	6.6	52	224	401	46	6.3	117
25	7.0	396	615	37	6.2	69	216	332	36.5	6.2	199
26	13.4	805	1443	46	7.6	72	588	1110	49	7.8	∞
						312	583	1045	46	7.3	
27	11.0	540	1190	57	6.3	72	320	796	62	6.7	372
						195	322	795	62	6.3	372
						333	288	710	62	6.3	372
28	10.5	627	1413	58	5.4	57	356	805	56	5.6	140
29	12.6	910	1550	43	5.9	71	480	875	47	6.3	263
						241	462	865	48.5	6.4	263
30	7.3	631	1000	38.5	6.4	66	370	591	39	6.4	140

Dog 26—not in shock.

∞ = survived.

Time of injection means time measured from the end of traumas.

during shock there is a high degree of variability in the level and stability of the blood pressure.

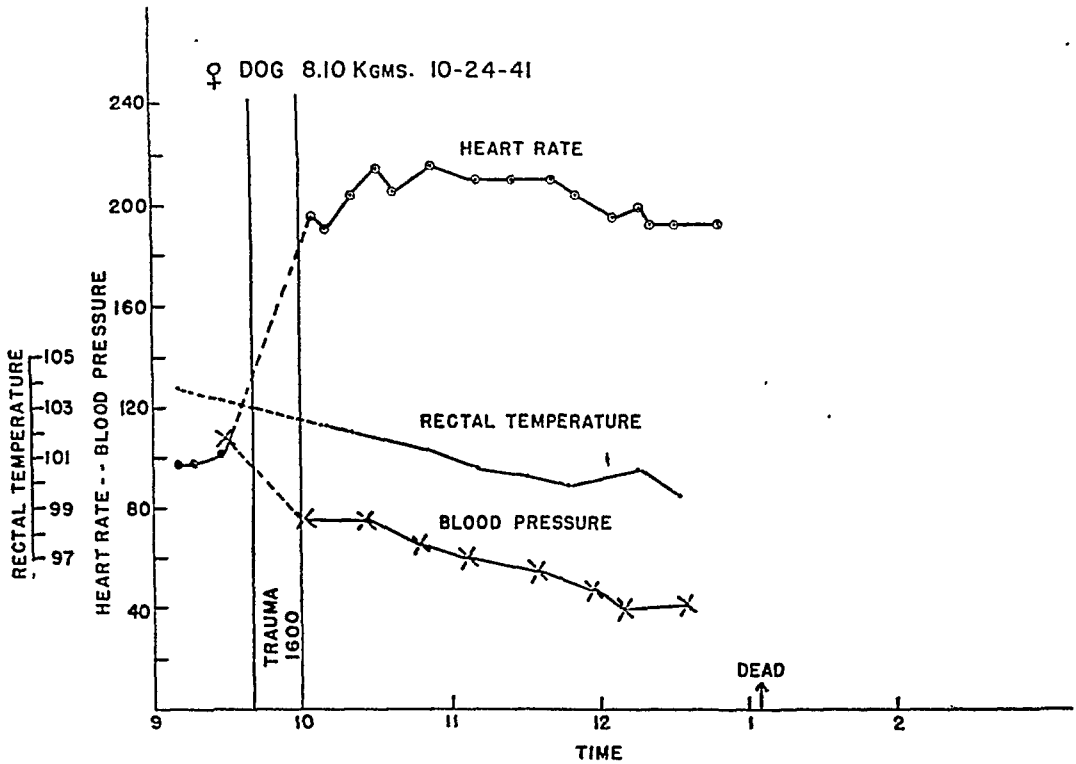


Fig. 4. The effect of muscle trauma on dog 17. In this animal the blood pressure fell progressively instead of being maintained at a plateau as in figures 1 and 3. The decrease in rectal temperature is greater than that shown by most of the animals.

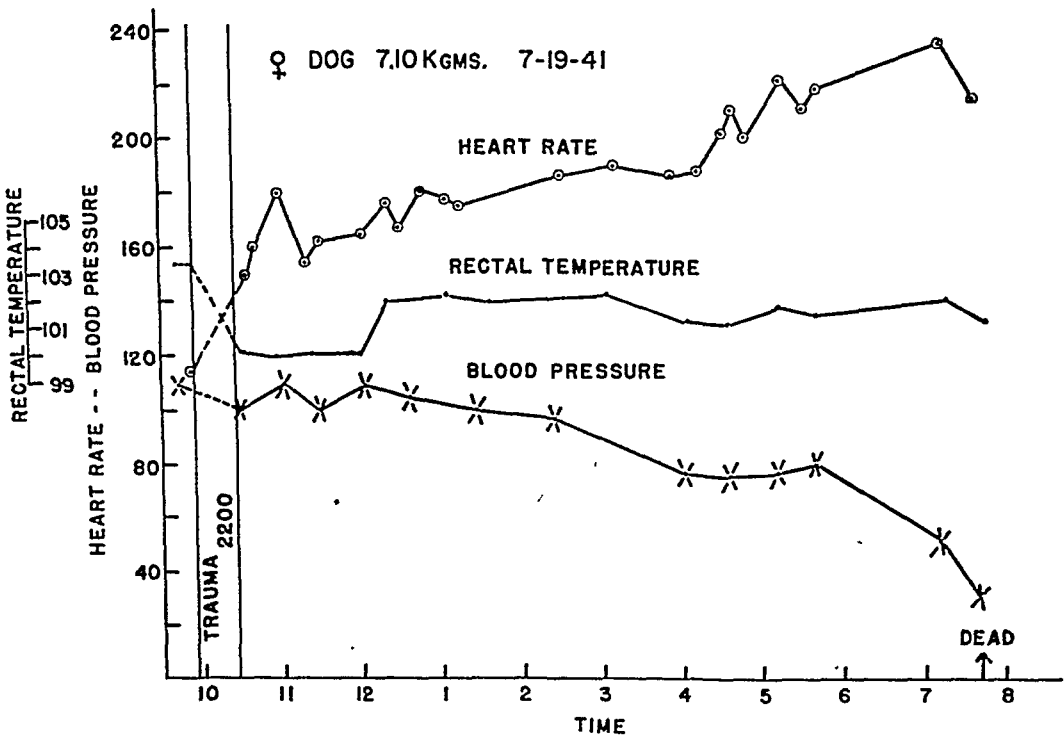


Fig. 5. The effect of muscle trauma on dog 3. In this animal the blood pressure fell and the heart rate increased progressively. The rectal temperature fluctuations were related to changes in room temperature.



Fluctuations in mean arterial pressure of 5 to 20 mm. Hg recurring in waves, were commonly seen after severe trauma. These were not in phase with the respiration and presumably arose from variations in the activity of the vasomotor center. It should be noted that in recording the blood pressure we took the average reading.

*Heart rate.* During the control period the heart rate usually fell to the normal range of 80 to 100 beats per minute as the dog became accustomed to the surroundings (see fig. 1). There were exceptions to this as shown by dog 26 (fig. 2) where the control rate ranged from 130 to 150 beats per minute. In one animal the control rate was 50. However, the average from all experiments of the last determination made just before trauma was 95 beats per minute. The administration of ether produced the usual tachycardia (19). After ether was discontinued the heart rates in a large fraction of the experiments fell to about 150 beats per minute but as the signs of shock developed, the heart accelerated again to more than 200 beats per minute (figs. 1 and 5). In other animals the heart rates remained high throughout the post-traumatic periods (fig. 4). During shock the heart rates of 23 of the 29 dogs ranged between 200 and 240 beats per minute. In the other 6 the rates varied from 170 to 190. The fate of the animal could not be predicted from the magnitude of the tachycardia. Whereas the peripheral pulse decreased in amplitude until it could scarcely be detected, the heart beat could be heard more distinctly and the force with which the apex was thrust against the chest wall became greater as shock developed. In all instances in which the heart rates were counted during the last few minutes of life we found a terminal bradycardia associated with a rapidly falling blood pressure (figs. 1 and 3).

*Body temperature.* The rectal temperature of the dogs after trauma ranged from 1 to 6°F. below the control level. In some experiments (see figs. 3 and 5) the fall in temperature appeared immediately after the trauma. In these the drop may at least in part be attributed to ether anesthesia (2). In other instances (see figs. 1 and 4) it seemed to be more definitely related to the development of shock. As one might expect, environmental temperature is a considerable factor. At low room temperature (20 to 22°C.) the rectal temperatures during shock were always markedly subnormal whereas at room temperatures above 26°C. the decrease might be only 1 or 2°F. On hot summer days the rectal temperature of dogs even in deep shock sometimes rose above the control level. It should also be noted in passing that we occasionally saw sudden upward changes in rectal temperatures during the post-traumatic period (see fig. 5). These may be a reflection of abrupt alterations in vasomotor tone or of redistribution of the blood to central regions resulting in a decreased heat loss without a corresponding decrease in heat production.

*Signs of vasoconstriction.* The progressive coldness of all the extremities and the dry lifeless appearance of the oral mucous membranes are among the most striking and consistent signs of the peripheral vasoconstriction. In the cutaneous areas the flow is soon completely shut off for as shock developed in our dogs it

was no longer possible to obtain capillary blood by incision of the skin.<sup>4</sup> The superficial veins in all parts of the body are so constricted that they disappear from view. Even large vessels such as the jugular vein carry only a trickle of blood and it is almost impossible to withdraw samples when the vein is successfully punctured. Such evidence demonstrates in a dramatic fashion how severely the vascular bed is restricted in shock.

*Respiration.* No measurements of respiration were made in the present study but it was apparent from inspection that the thoracic excursions became exaggerated in all the animals as signs of shock developed. In the dogs that survived less than  $2\frac{1}{2}$  hours a marked increase in respiration occurred almost at once after the injury. Toward the end the breathing showed pronounced irregularities and gasping was common in the terminal stages. The changes in pulmonary ventilation and blood gases will be reported elsewhere (4, 45).

*Thirst and vomiting.* The presence of intense thirst after trauma is evidenced by the fact that in all instances where dogs in shock were offered water they drank large amounts with great eagerness. One example may be cited. Dog 23, one of the animals that showed characteristic signs of severe shock, but survived, was kept on the animal cradle for  $5\frac{1}{2}$  hours after the injury. At the end of this period the heart rate was 225 beats per minute, and the blood pressure 70 mm. Hg and yet when put on the floor the dog walked about without much difficulty. He was then offered a pan of water and at once drank several hundred cubic centimeters without stopping. A few moments later he vomited. It should be stated that in the 29 dogs in shock we saw only two instances of spontaneous vomiting, except when the dogs were given water. These two occurred late in the shock syndrome. Our impression from these and many subsequent experiments is that vomiting is a relatively rare phenomenon in experimental traumatic shock in dogs, and that it is brought on by the ingestion of fluid at a time when the circulation to the gastro-intestinal tract is so much reduced that the fluid cannot be absorbed.

During the terminal stages of shock, the animals frequently showed tonic muscular contractions similar to the straining movements of defecation. These have the merit of increasing intra-muscular pressure and assisting the venous return.

*Central nervous depression.* At least 60 per cent of the shocked dogs showed unmistakable signs of a progressive central nervous depression. As shock developed occasional movements of the dogs became fewer and the animals no longer responded to petting. If the dog had shown an earlier tendency to vocalize, this gradually ceased. Only rarely was the general apathy interrupted by periods of restlessness. During the period of depression there was a distinct increase in the sensory threshold, as shown by the fact that the skin over the

<sup>4</sup> In this connection it may be noted that we found as others have, that the erythrocyte count of the cutaneous capillary blood was very much higher than in central venous or arterial bloods collected at the same times. The difference was of the order of 2 to 3 million cells per cu. mm.

femoral artery could be incised and the femoral artery dissected without so much as attracting the dog's attention. This observation is of some interest for numerous military surgeons have reported that severely wounded men suffer little or no pain (5). At the same time the pupillary response to light became sluggish and in some animals in shock it could not be elicited. Shortly before death the apathy gradually changed into coma.

Among the dogs which had few signs of central depression only a couple showed a persistent tendency to be restless throughout the period of shock. It is noteworthy that the animals which displayed any degree of restlessness after trauma were the ones that struggled and vocalized when first placed upon the animal board, and by comparison with this earlier behavior one would certainly conclude that they were less restless during shock. Since such dogs were quieted easily by gentle stroking and petting, it may be inferred that anxiety rather than discomfort was responsible for the restlessness.

*Animals recovering from shock.* As stated above 4 of the 29 dogs that exhibited the full picture of severe shock recovered spontaneously and survived. All had cold feet, pale and cold oral mucous membranes, and constricted jugular veins, from which samples could be drawn only with great difficulty. Dog 13 was a rather remarkable case. In this animal the blood pressure was only 60 mm. Hg for at least  $7\frac{1}{2}$  hours after the injury (control 142 mm. Hg). The post-traumatic heart rates of the 4 surviving dogs ranged from 170 and 220 beats per minute, and three had heart rates of over 200 beats per minute. After injury the rectal temperatures fell 2 to 4°F., but they returned to the control values during the state of shock. Changes in the general condition of the surviving animals were marked and readily detected. After passing through a period of general depression, apathy disappeared and the dogs seemed quite normal and responsive. This improvement was not necessarily associated with a marked change in blood pressure.

When it became quite apparent that a dog was recovering from shock it was placed in a cage where water was available. During the night dog 13, the one with the persistent low blood pressure after trauma, ingested 780 cc. of water and excreted 400 cc. of urine. Twenty-nine hours after injury the blood pressure was 100 mm. Hg (control, 142) and the heart rate was 170 beats per minute (control, 68). The rectal temperature had returned to the control value (101°F.). Dog 16 was also observed the day after the injury. The blood pressure which during the  $6\frac{1}{2}$  hours following trauma varied between 80 and 90 had returned to 120 mm. Hg (control, 118). The heart rate had declined from 220 beats per minute to 156 (control, 79) and the rectal temperature was 102°F. (control, 101°F.).

*Discussion.* Although the present report is concerned only with observations made on 30 animals, it should be stated that subsequent investigations carried out in this laboratory on many other traumatized dogs have confirmed in every respect the descriptions given above.

The individual variations shown by the different traumatized dogs indicate that it is impossible to select any one of the signs or symptoms and to state that

the appearance of this alone signifies the presence of traumatic shock. Nevertheless, certain phenomena are shown by the majority of the animals (fig. 6) and when taken together these signs may be regarded as evidence of shock. Thus, all of the shocked dogs had cold extremities, pale cold oral mucous membranes, constriction of superficial veins (e.g., jugular) in which the blood was dark and from which samples could be drawn only with greatest difficulty. All of the shocked dogs had arterial blood pressures which were lower than the control pressures and heart rates which were considerably higher than their resting values. Seventy-nine per cent of the shocked animals had heart rates which exceeded 200 beats per minute. Eighty-six per cent showed a fall in rectal

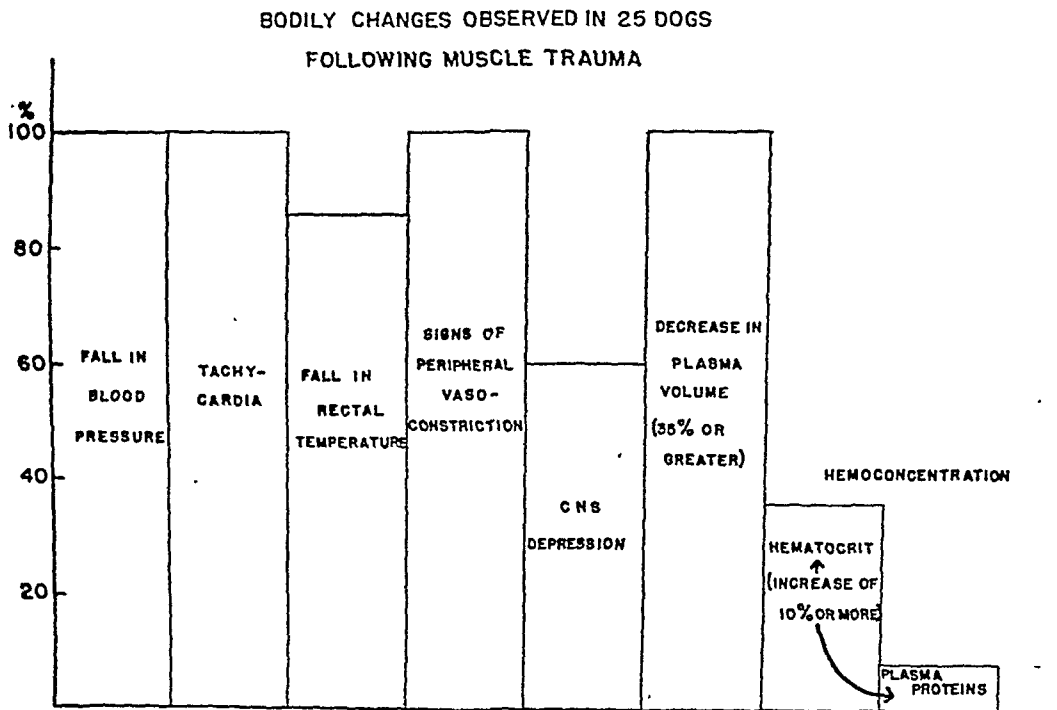


Fig. 6. Shows the frequency with which the various criteria of traumatic shock appeared in the 25 fatally shocked dogs studied in section A.

temperature and at least 60 per cent exhibited definite signs of central nervous depression. That the above responses to muscle trauma reproduce in the dog the syndrome of traumatic, surgical or secondary shock, as it occurs in man, is shown by the fact that the changes which occur in injured dogs are identical with those observed in man (5).

In man, extensive laceration of muscle without injury to bones or to any vital structure or organ produces shock (6). Under these circumstances the injured muscle becomes a dark brown, crushed matter without evidence of striation or vitality (7). This statement is a good description of the appearance of the injured tissues in the dogs subjected to muscle contusion.

Human traumatic shock is usually associated with a lowered and falling arterial blood pressure (5, 20). Nevertheless, severe shock may occur in the

presence of a reasonably high arterial pressure (8, 9, 10, 11). Similar observations have been made on our dogs. The failure of blood pressure to be a reliable index of the state of shock illustrates the hazard of studying experimental shock under anesthesia where almost the only objective criterion is this measurement. Our experiments suggest that a blood pressure below 60 mm. Hg results in death. We have seen only one animal survive an arterial pressure of 60 mm. Hg maintained for several hours. This agrees with Cowell's (12) observations on wounded soldiers.

Most writers state that the heart rate accelerates in human traumatic shock. According to Keith (8) the heart rate is higher, the greater the blood volume reduction. Cannon's (5) figures show that on the average the heart rate is faster at low than at high systolic blood pressures. On the other hand, Evans et al. (20) are not impressed with the value of the pulse rate as an index of traumatic shock. In the shocked dog the magnitude of cardiac acceleration is greater than in man, but the qualitative response to injury is in general that reported by Keith (8) and Cannon (5). The terminal bradycardia is probably the result of severe hypoxia on the vagal center (see 13).

Certain observations such as the pale mucous membranes, the cold extremities, the constriction of veins and the dark color of the venous blood which we noted consistently in the dogs stand out in all descriptions of wound shock (5).

A decrease in body temperature of shocked man has been reported by a number of observers. Cannon (14) recorded mouth temperatures between 87 and 95°F. Archibald and McLean (15) studied 12 cases in which the body temperature was as low as 92°F. According to Weil (16) rectal temperatures in human shock do not exceed 37°C., and in severe cases may be 31°C. or even lower. Decreases in rectal temperature of as much as 6°F were seen in some of our animals. However, the temperature of the shocked dog is influenced by the room temperature, being low when the environment is cold and falling very little or even rising if the experiment is carried out on a hot summer day.

Cannon (14) states that shivering almost never occurs in human shock. None of our dogs shivered.

Cannon (5) reported a more rapid but probably a more shallow respiration in human traumatic shock. A more detailed statement is made by Blalock (18) who reports that the rapid respiration of the early stages of shock is succeeded by deeper and slower breathing, as stupor and finally coma occur. Pronounced irregularity of respiration and gasping occur only when the patient becomes moribund. Our observations on dogs agree with this description. Actual measurements indicate that the pulmonary ventilation is increased during traumatic shock in both man (11, 17) and dog (4).

The majority of our animals showed unmistakable signs of central nervous depression. As shock developed the occasional movements of the animal became fewer and he no longer seemed aware of his environment. The apathy which was sometimes interrupted by periods of restlessness gradually developed into coma some time before death. Two dogs had a tendency to be restless throughout the period of shock. The similarity to human cases of shock is striking. Descriptions of wound shock or surgical shock (5, 18) mention apathy,

stupor and dulled sensations as prominent features in the clinical picture. Cannon (5) reports restlessness and anxiety in some patients.

There is no doubt about the presence of intense thirst in the shocked dogs, and according to Cannon (5) this is the universal complaint of wounded soldiers. Furthermore, all dogs that were given water when in deep shock vomited shortly afterwards. Like patients in shock they have difficulty retaining fluid given by mouth.

Cowell (12) has called attention to the profuse sweating which accompanies severe wounds. He reports that wounded men may complain more of sweating than of the wound itself. On the other hand, Evans et al. (20) state that there was no sweating in their patients during traumatic shock. In dogs the sweat glands are rudimentary or absent except upon the pads of the feet. We did not observe sweating of the footpads.

The supine position in which the dogs were maintained upon the animal board is admittedly unnatural. In this laboratory we have seen 3 dogs which survived shock suddenly expire the following day when they were again placed upon the animal board. We do not know how great a rôle this factor may play in our experiments.

**B. TRAUMATIC SHOCK PRODUCED BY BULLET WOUNDS.** The production of shock by contusing muscles is, of course, a highly artificial procedure. For this reason it seemed important to compare the results with those produced by a form of injury actually encountered in warfare.

Five experiments were carried out to study the effects of gunshot wounds. In dogs under ether anesthesia, 5 shots (0.22 long, hollow point cartridges), placed in the flexor muscles of each hind limb, were apparently as effective in causing shock as the muscle bruising technique used in the experiments described in the preceding section (fig. 7). The bullets leave small clean holes in the skin and cause only a trivial amount (1 to 5 cc.) of external hemorrhage. Considerable swelling, however, appears at once, accompanied by discoloration of the skin similar to that seen after severe bruising. Autopsy reveals extensive laceration of the muscle surrounding the path of the bullet and leaving cavities the size of a large egg, filled with bloody fluid.

These experiments demonstrate that the physiological signs which in man are recognized as characteristic of traumatic shock can be reproduced experimentally either by mechanical contusion or gunshot wounds.

**C. BLOOD VOLUME.** During the war of 1914-1918 it was found that the blood volume was reduced in human traumatic shock (8, 21). Moreover, the diminished blood volume was recognized as bearing a definite relation to the clinical condition of the patient. Evidence from bleeding volume (29) as well as from plasma volume measurements with dilution methods (30) had also demonstrated a large decrease in blood volume in experimental shock in animals. Although certain questions have been raised as to the accuracy of these early blood volume determinations, Freeman (23) stated in 1943 that, "it is generally agreed by all of those who are working in the field of shock that loss of blood, plasma or body fluid is the most significant initiating factor in the production of shock."

The decrease in circulating volume indicates that fluid has been lost into extra-

vascular regions. The problem of what Cannon (5) called the "lost blood" has occupied the attention of many investigators. Blalock and others (see 18) have suggested that the fluid loss in the region of injury is sufficient in amount to account for the reduction in the circulating volume seen in traumatic shock. Unfortunately, these workers made no measurements of blood volume. Hence, there was no way of knowing whether in their experiments local tissue swelling accounted for part or all of the blood volume reduction.

Before we undertook the present investigation in 1941 the prevailing opinion had been that in shock the circulating blood volume progressively decreases as the result of a generalized increase in capillary permeability. Indeed, as

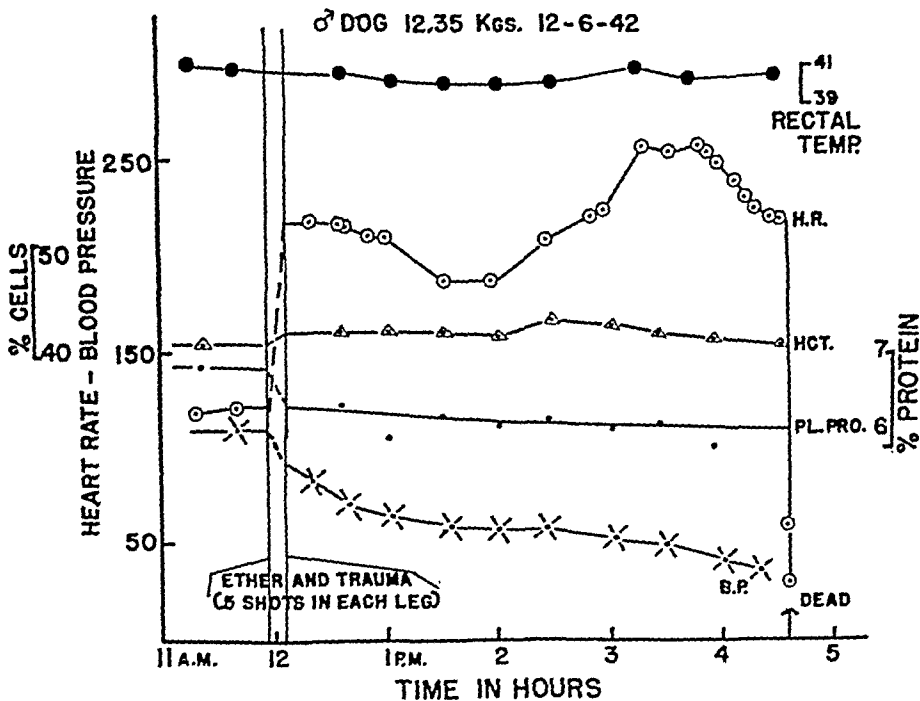


Fig. 7. A typical experiment of traumatic shock produced by gun-shot injury. Five 0.22 calibre long hollow point bullets were placed in the flexor muscles of each hind limb. The clinical condition of the animal was in every respect identical with that produced by muscle contusion.

recently as 1943, shock was defined "as the clinical condition characterized by progressive reduction in circulating blood volume due to increased capillary permeability" (23). According to this concept plasma escapes through the capillary wall and the concentration of the red blood cells increases. The resulting hemoconcentration was considered an indication of the early stages of shock (24, 25, 26) and it was on this basis that Moon differentiated between hemorrhage and shock. Freeman (23) used the presence or absence of hemoconcentration to distinguish between conditions in which plasma only is lost and conditions in which loss of both red cells and plasma occurs.

In the study of traumatic shock a method for estimating whole blood volume would be of considerable value not only as an index of the circulatory deficit, but also as a measure of the volume required in replacement therapy. The

usual method is to determine plasma volume and to calculate the total volume from this measurement and the hematocrit reading. However, this technique was rejected by several investigators who reported that in well-developed shock the dye method of measuring plasma volume gave unreliable results (23, 27, 28). We have found that when proper precautions are taken and careful technique is used the plasma volume can be measured as conveniently and accurately in dogs in traumatic shock as in normal dogs. Our methods and results are described below.

*Methods.* Ordinary mongrel dogs, weighing between 8 and 20 kgm. were used. The animals were not fed after noon of the day preceding the experiment but they had free access to water until they were placed on the animal board.

After one half-hour of rest the determination of the control plasma volume was started and blood samples were taken at frequent intervals for a one hour dye curve and for plasma protein and hematocrit readings. Ether was then administered and the thigh muscles were contused according to the method described in the first section of the present paper. At various times after trauma the blood volume measurements were repeated. In some animals several determinations were made.

Plasma volume was measured with the dye dilution method, using the technique developed by Gregersen et al. (31, 33, 34). The dye, T-1824, was injected into the femoral or jugular vein. For the control blood volume the dye tinged samples were drawn from the jugular vein. After trauma they were drawn from the femoral artery. The serum dye concentrations were read with a Koenig-Martens visual spectrophotometer (31). When, during the course of a dye curve, the plasma protein concentration varied by more than 0.2 gram per cent from the control value, dye concentrations were corrected with reference to the control protein concentration as described elsewhere (42).

The plasma protein concentration of each blood sample was measured with an Abbe refractometer (35). Hematocrit values were determined by centrifuging heparinized blood samples in Wintrobe tubes for 30 minutes at 3000 r.p.m. (radius 13 cm.).

In the determination of plasma volume after muscle trauma-certain precautions must be observed. Muscle trauma invariably causes some hemolysis, but the amount of free hemoglobin in the blood stream changes very gradually (44). Hence, the dye concentration can be determined spectrophotometrically in the serum *provided the blood samples are collected without additional hemolysis*. During shock all determinations of dye, serum proteins and hematocrit must be made upon arterial samples. Venous samples from dogs in profound shock may give bizarre results.

The total blood volume was calculated from the formula:

$$\frac{\text{plasma volume}}{100 - (\text{hematocrit} \times 0.96)} \times 100$$

The factor 0.96 is the correction for the plasma trapped with the erythrocytes. The blood volumes, calculated from the plasma volume as determined with T-1824, and the hematocrit reading have recently been compared with those



obtained by measuring the plasma volume with T-1824 and the red cell volume with CO (36, 47). The results agree to within 5 or 10 per cent not only in normal

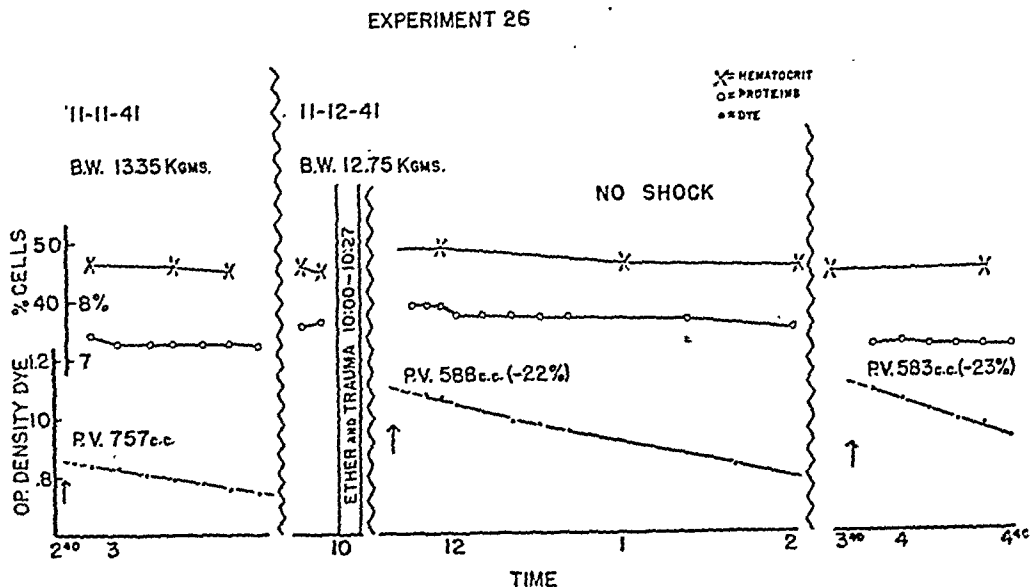


Fig. 8. The effect of muscle trauma upon the dye disappearance curves, the plasma protein concentrations and the hematocrit values in dog 26.

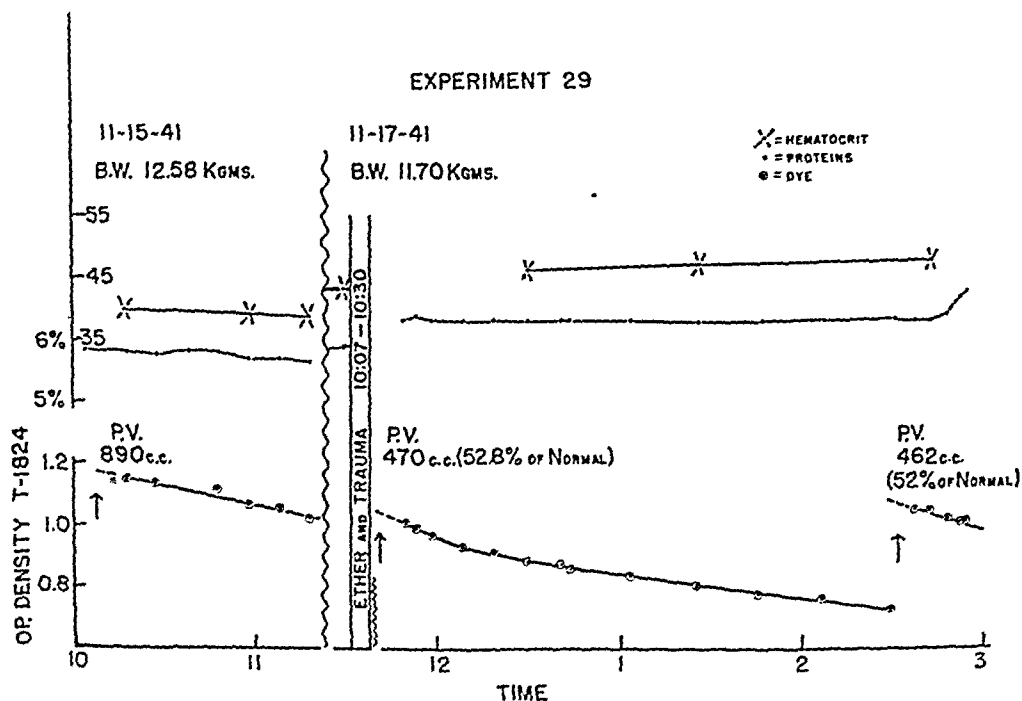


Fig. 9. The effect of muscle trauma upon the dye disappearance curves, the plasma protein concentrations and the hematocrit values in dog 29. The animal died 4 hours and 23 minutes after trauma.

and splenectomized dogs, but also in animals in which the blood volume has been greatly reduced by muscle trauma or hemorrhage.

*Results.* Typical time-concentration curves of T-1824 before and after mild and severe muscle trauma are shown in figures 8 and 9, respectively. Technically, the curves obtained after muscle trauma are as good as those carried out during the control period. It should be mentioned that after muscle trauma (and also after hemorrhage) the disappearance rate of the dye is greater than in the control determinations.

Comparison of the percentage reduction in plasma volume produced by muscle trauma with the percentage reduction in calculated blood volume shows that these rarely agree. The reason for this discrepancy is that after muscle trauma

### TRAUMA - NORMAL DOGS

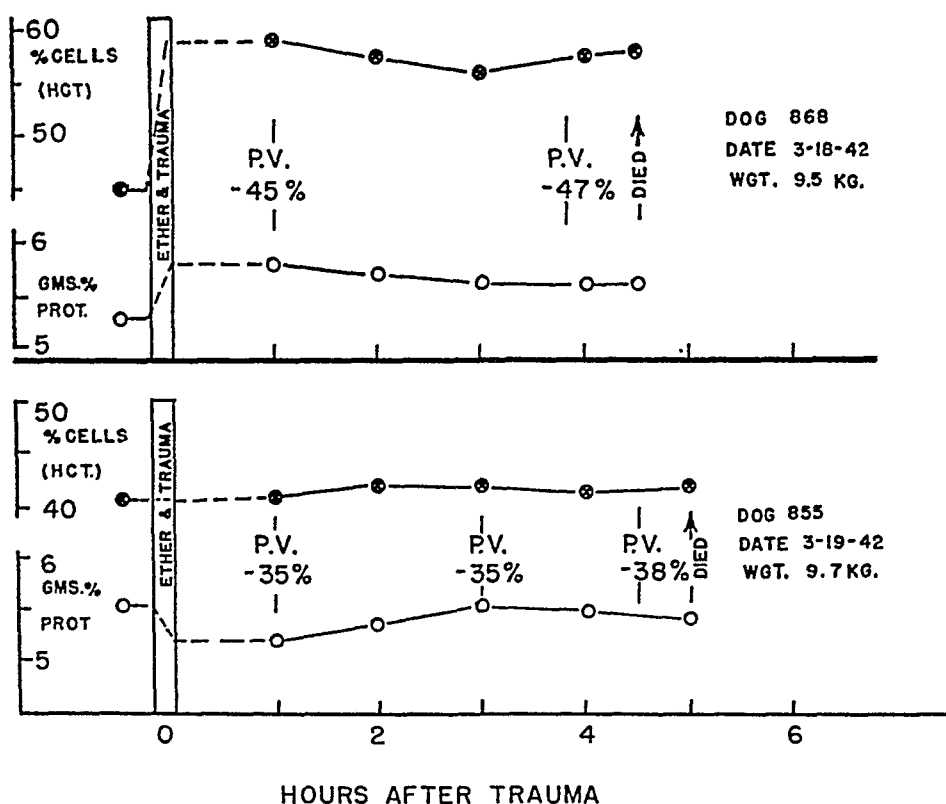


Fig. 10. The effect of muscle trauma upon the plasma protein concentrations and the hematocrit readings of 2 normal dogs (dogs 33 and 34).

the percentage of red cells varies in the different experiments from 8 hematocrit units below to 16 hematocrit units above the control values (figs. 7, 8, 9, 10). On a frequency distribution curve the mode was an increase of 4 hematocrit units. These changes in the hematocrit reading are probably related to changes in the size of the spleen, for ether anesthesia (37) and blood volume reduction (38) are known to produce splenic contraction. Moreover, in splenectomized dogs muscle trauma produces little or no change in the hematocrit values (fig. 11).

The blood volume was measured before and after muscle trauma in 26 of the 30 dogs studied in section A (fig. 12). All except dog 26 (see fig. 8) were in shock. Of the 25 animals in which the blood volume was estimated during shock, 4 survived (dogs 6, 13, 16, 23 table 1). Fatal shock was produced in 20

of 21 dogs in which the blood volume reduction was greater than 30 per cent. Only 1 of the 5 animals in which the decrease in blood volume was less than 30 per cent died. There is no indication in figure 12 of any tendency for the blood volume reduction measured some hours after muscle trauma to be greater than that determined within one hour of injury. In 3 of the 4 experiments (dogs 23,

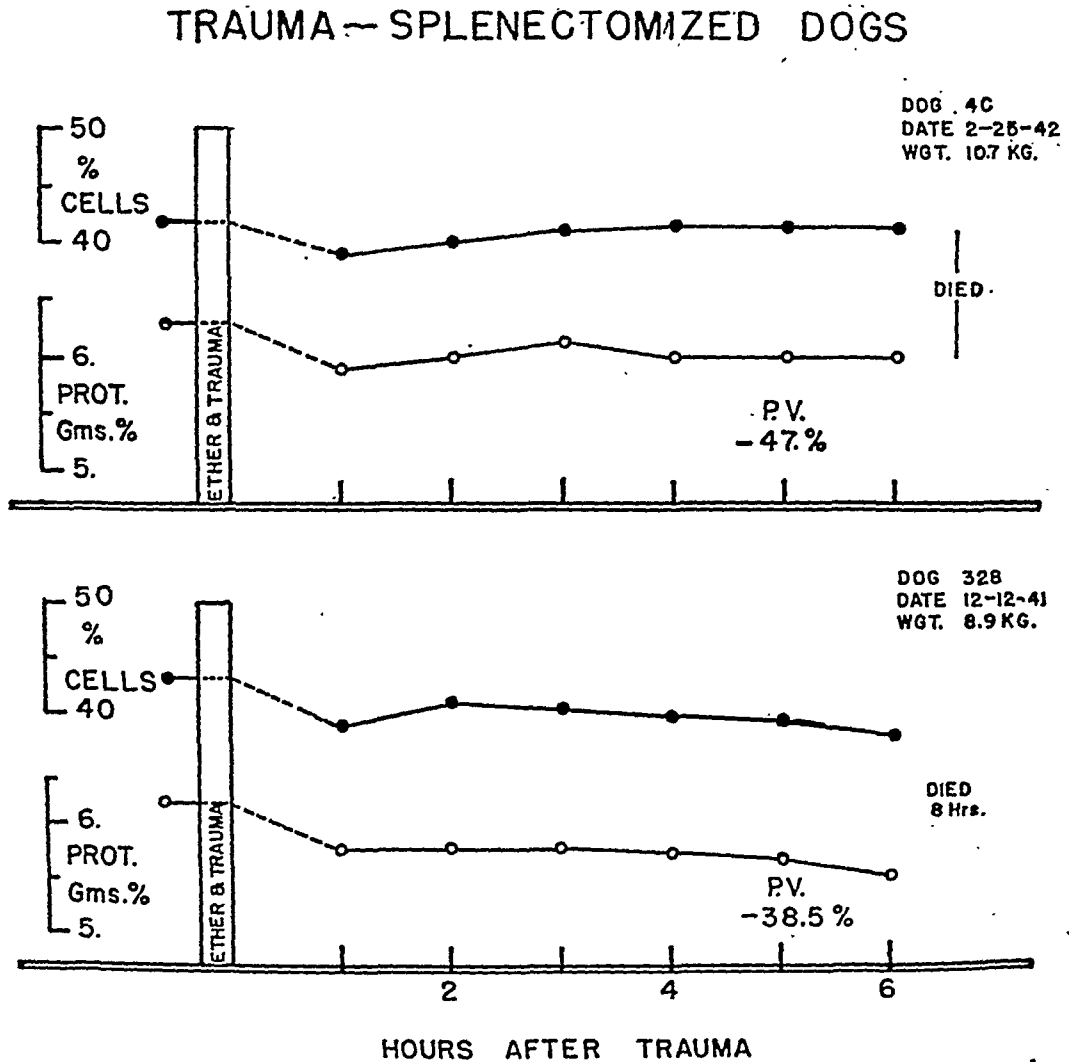


Fig. 11. The effect of muscle trauma upon the plasma protein concentrations and the hematocrit readings of 2 splenectomized dogs.

26, 27, 29) in which more than one measurement of blood volume was made after muscle trauma, the blood volume reduction remained constant. In the fourth animal (dog 27) the first and second measurements after injury agreed, but the third estimation showed a blood volume decrease which in terms of the control volume was 7 per cent lower than the previous determination.

The maintenance of a decreased but a constant level of blood volume after muscle trauma was unexpected and seemed of sufficient importance to merit

more extensive study. For this reason the blood volume of each of 17 additional dogs was determined repeatedly after injury (fig. 13). In only 4 of the 21 animals studied (figs. 12 and 13) did the blood volume after injury show a further decrease amounting to more than 5 per cent. The findings in one of these animals (dog 27) have been mentioned above. Dog 33 shows a progressive decrease in both plasma volume and blood volume. In this animal the blood volume was 631 cc. 37 minutes after muscle trauma. The volume decreased to 550 cc. 104 minutes later and to 511 cc. 252 minutes after injury. The blood volume of dog 44 was reduced from 610 cc. before trauma to 438 cc. 31 minutes after injury and to 398 cc. 134 minutes later. The control blood volume of dog 46 was 1215 cc. Thirty-five minutes after injury the blood volume was 1040 cc. This decreased to 968 cc. at 220 minutes and 938 cc. 372 minutes after muscle trauma. The

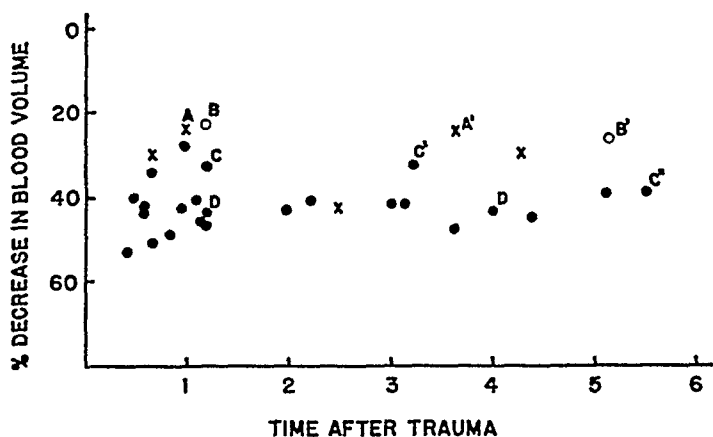


Fig. 12. The effect of muscle trauma upon the blood volume of 26 of the 30 dogs studied in section A. The per cent decrease in blood volume is plotted against the time after injury at which the plasma volume and hematocrit reading were determined.

x Survived  
 ● Died  
 ○ Muscle trauma but no shock

} Shock

results obtained from the remaining 17 animals indicate that the reduction in blood volume, measured 30 minutes after muscle trauma, shows no further decrease. It should be noted that no correction has been made for the blood removed as samples.

The constancy of the plasma and blood volume after trauma is reflected in the constancy of the hematocrit readings (figs. 7, 8, 9, 10). After muscle trauma the hematocrit values are usually greater than during the control period but they are maintained at the new level with little change for several hours. The plasma protein concentration varies after muscle trauma from a decrease of 0.8 to an increase of 1.0 gram per cent. As in the case of the hematocrit values the new plasma protein level remains relatively constant for some time (figs. 7, 8, 9, 10).

Four dogs which survived muscle trauma were placed in cages and allowed to drink water freely. Approximately 24 hours later the volume determinations

were repeated. The blood volume of dog 13, which was reduced to 60 per cent of the control value, had recovered to 71 per cent the next morning. In the case of dog 16 the blood volume, which was 70 per cent of the control value, had returned to 75 per cent the following day. After muscle trauma the blood volume of dog 32 was 60 per cent of the control determination. The next morning the

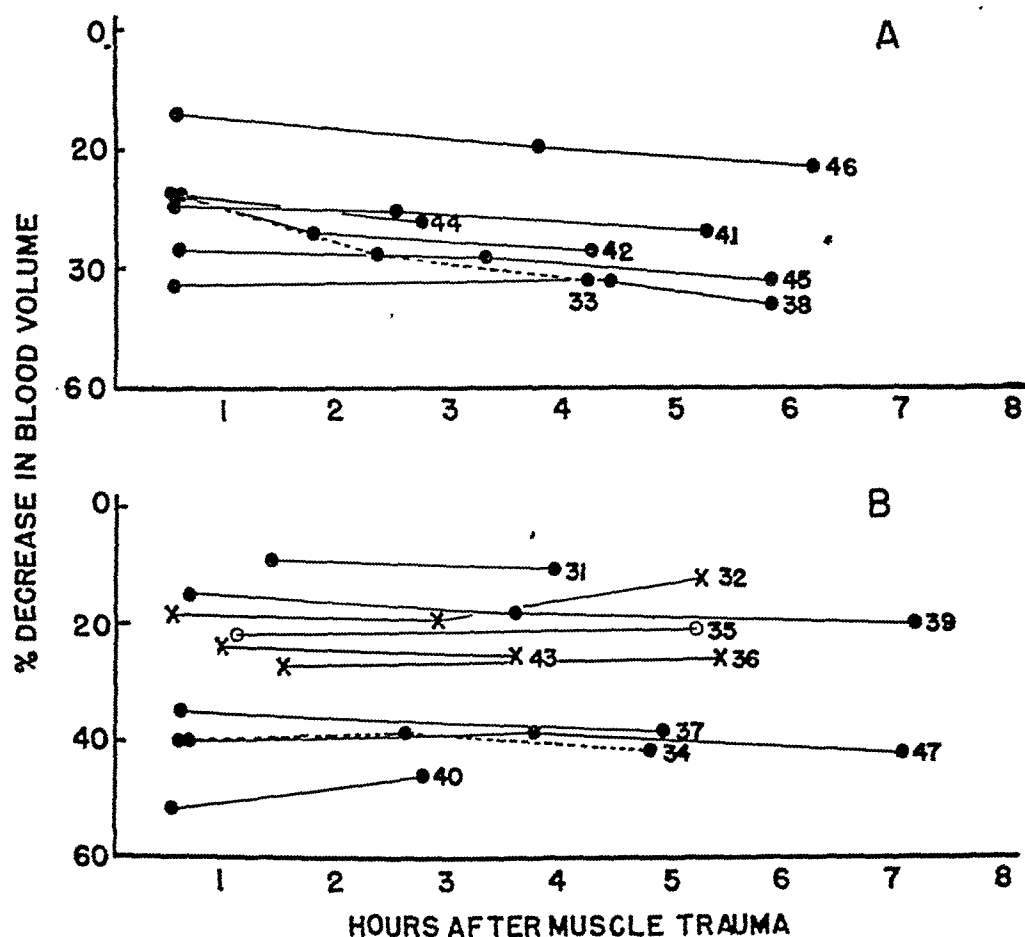


Fig. 13. The effect of muscle trauma upon the blood volume of 17 dogs. At various times after injury repeated determinations of plasma volume and the hematocrit values were made upon each animal. The volumes were not corrected for the blood samples taken.

× Survived

● Died

○ Muscle trauma but no shock. B. Ten dogs in which the blood volume showed no evidence of decrease on repeated determinations.

Shock

A. Seven dogs in which the blood volume was lower on the second or third determination.

volume had increased to 86 per cent. In the fourth animal, dog 35, the volume was reduced to 78 per cent and over night returned to 86 per cent of its control volume. The increased blood volume was brought about by the entrance of fluid relatively poor in protein, for the plasma protein concentration decreased. The fall in the hematocrit reading is greater than can be accounted for on the basis of dilution and probably indicates some relaxation of the spleen with withdrawal of red cells from the active circulation. The results suggest that the

recovery of blood volume after muscle trauma is a relatively slow process. The changes during recovery have been studied more extensively by Holmes (3).

We were somewhat puzzled by the appearance of fatal shock in certain animals with small reductions in blood volume (fig. 13). One of these, dog 31, died 3 hours and 56 minutes after trauma with a blood volume reduction of only 13 per cent, although the plasma volume was down 27 per cent. In another animal, dog 39, in which death occurred 9 hours and 53 minutes after injury the blood volume was reduced by 20 per cent, the plasma volume by 29 per cent. The only unusual feature shown by this animal was the high blood pressure which did not fall below 100 mm. Hg until one hour and 40 minutes before death. At least two factors, on which good evidence has subsequently been presented, namely, infection (32) or afferent nervous effects (40, 41) may have contributed to the fatal shock in these instances where the blood volume was only slightly reduced.

*Discussion.* It is apparent from examination of the dye curves obtained on dogs after trauma (see figs. 8 and 9) that the injury and shock do not fundamentally alter the validity of this procedure for determining plasma volume. Actually the mixing time may be shorter in dogs after the injury than during the control period. Although the disappearance rate of the dye is usually higher after trauma this cannot be taken as evidence of actual increase in general capillary leakage. The same thing occurs when the blood volume is reduced by simple hemorrhage whether or not the animal is in shock. Redistribution of blood after a loss in volume may simply reduce the fraction of blood passing through regions of low capillary permeability (muscles) and leave a relatively larger fraction passing through internal organs (liver) where the leakage is normally high. At any rate the extrapolation of the dye curves corrects the determination of plasma volume for any differences in the disappearance rates. It should be noted that in man a large reduction in blood volume caused by skeletal trauma or simple hemorrhage has little, if any, effect on the rate of disappearance of the dye, even if the patient is in severe shock (20, 42). The difference between dog and man in this respect may arise from the fact that in man the compensatory vasomotor responses and redistribution of blood is not so drastic as in the dog (4). The results in man show quite clearly that the rate of disappearance of T-1824 from the blood stream is not related to the degree of shock, but varies with the extent of capillary bed that is damaged.

Muscle trauma which is severe enough to reduce the blood volume by 30 per cent or more invariably produces shock and the shock is usually fatal (figs. 12 and 13, tables 1 and 2). In some instances fatal shock is produced when the decrease in blood volume is less than 30 per cent. Repeated blood volume determinations after the thighs have been contused show that in this form of experimental trauma the development of shock is not associated with a progressive decrease in the blood volume. The absence of progressive decrease in volume is demonstrated also by the constancy of the hematocrit values and plasma protein concentration (figs. 7, 8, 9, 10, 11) as shock develops. These results refute the concept, at one time widely accepted, that the fundamental cause of shock is a general increase in capillary leakage.

Subsequent investigations in this laboratory have brought forth convincing

TABLE 2  
*Blood volume measured repeatedly after muscle trauma*

DOG. NO.	WT.	CONTROL				TIME OF DYE INJ.	POST TRAUMA				SURVIVED
		Plasma vol.	Blood vol.	Hct.	Plasma Prot.		Plasma vol.	Blood vol.	Hct.	Plasma Prot.	
	kgm.	cc.	cc.	%	gm. %		cc.	cc.	%	gm. %	
31	10.4	577	864	34.5	5.4	85	436	770	45	5.6	3 hrs. 56 min.
						266	422	755	46	5.7	
32	10.8	611	1040	43	5.2	32	370	852	59	5.7	∞
						173	350	843	61	5.7	
						315	381	918	61	5.5	
33	9.5	521	880	42.5	5.4	37	277	631	58.5	5.7	4 hrs. 35 min.
						141	249	550	57	5.7	
						252	229	511	57.5	5.7	
34	9.3	500	897	46.5	5.2	42	324	535	41	5.2	5 hrs. 24 min.
						157	324	547	42.5	5.5	
						282	310	516	41.5	5.2	
35	14.0	850	1435	42.5	6.5	67	500	1115	57.5	7.0	∞
						311	510	1125	57	6.5	
36	10.3	625	963	36.5	4.9	92	407	700	43.5	5.3	∞
						327	445	712	39	4.9	
37	10.3	501	920	47.5	6.0	37	335	600	46	5.2	7 hrs. 45 min.
						297	320	563	45	5.1	
38	10.4	508	946	48.2	7.1	33	306	543	45.4	6.5	6 hrs. 19 min.
						266	325	549	42.5	6.4	
						350	302	506	42	6.3	
39	10.5	480	933	50.5	6.8	42	352	797	58.2	6.9	9 hrs. 53 min.
						216	341	761	57.5	6.7	
						429	344	748	56.3	6.7	
40	13.9	801	1388	44	6.2	33	332	664	52	6.1	4 hrs. 7 min.
						162	388	746	50	6.1	
41	14.8	906	1452	39.4	6.5	34	520	1010	50.6	7.5	8 hrs. 15 min.
						150	520	1005	50.3	7.5	
						314	505	954	49	7.4	
42	11.1	442	779	45.1	5.3	107	246	516	54.5	5.3	5 hrs. 2 min.
						257	219	457	54.3	5.3	
43	8.5	512	726	31	4.8	67	358	536	34.5	4.5	∞
						225	358	546	35.5	4.4	
44	5.7	390	610	37.5	5.0	31	291	438	35	4.4	4 hrs. 5 min.
						165	264	398	35	4.3	
45	7.6	429	775	46.5	5.2	35	273	490	46	5.1	8 hrs. 16 min.
						198	276	482	44.5	4.7	
						350	270	447	41.5	4.7	
46	10.9	626	1251	51.6	5.0	35	481	1040	56	4.9	9 hrs. 17 min.
						220	452	968	55.5	4.7	
						372	452	938	54	4.6	
47	8.3	462	848	47.4	5.9	36	283	506	47	5.7	8 hrs. 37 min.
						226	286	513	46	5.3	
						424	286	504	45	5.0	

Of the four surviving dogs (∞) only dog 35 was not in shock.

additional evidence against the theory of generalized capillary leakage (22, 43), and have shown that loss of fluid occurs only into the injured area. This loss is fully accounted for by the decrease in blood volume and by the mobilization of fluid from uninjured areas.

Although ether was administered only for a short period, this does not rule out the possibility that it modified the results of the experiments. In a normal dog the ether would be almost completely eliminated within 20 to 30 minutes. The situation in the traumatized dog is not clear, for the slowed blood flow in these animals would be expected to decrease the rate at which ether leaves the body, whereas the increased pulmonary ventilation should favor its elimination (4). At least so far as we could judge from superficial examination, the recovery from ether anesthesia did not seem to be slower in the traumatized dogs than it is in the normal animal. With a view to eliminating the ether factor altogether we carried out several experiments on chronic spinal dogs (level of 10th to 12th thoracic segments) in some of which the lumbar sympathetic chains had also been removed. In these animals the thigh muscles were traumatized without general anesthesia and the plasma volumes determined again immediately (10 to 15 min.) after the trauma. Here also the results showed that the blood volume reduction occurred at the time of injury. For many hours thereafter the volume did not change appreciably. So far then as the blood volume changes are concerned, the ether did not appear to have modified the outcome.<sup>5</sup>

This conclusion receives further support from more recent experiments on hemorrhagic shock (39) in which it has been shown that the L. H. 50 (residual blood volume giving 50 per cent mortality) is not altered by carrying out the bleeding during a brief period of etherization.

#### SUMMARY

1. The clinical signs shown by dogs in which the thigh muscles have been contused are identical with those which in man constitute the syndrome of secondary shock. These consist of increased heart rate, decreased body temperature, various signs of vaso-constriction, changes in respiration and evidence of central nervous depression (figs. 1 to 6). As in humans, dogs in shock do not shiver even though the body temperature may be depressed several degrees. In both species thirst is present and ingested fluid is usually vomited. In the dog it has been possible to reproduce all of the signs of clinical shock with the exception of sweating (fig. 6).

2. The physiological signs which in man are recognized as characteristic of traumatic shock can be reproduced experimentally in the dog by gunshot wounds (fig. 7).

3. Muscle trauma and shock do not fundamentally alter the validity of the dye dilution method for the measurement of plasma volume (figs. 8 and 9). Muscle trauma which is severe enough to reduce the blood volume by 30 per cent or more

<sup>5</sup> The details of the experiments on traumatic shock in spinal dogs will be described in detail elsewhere.



invariably produces shock, and the shock is usually fatal (figs. 12 and 13, tables 1 and 2). Fatal shock sometimes occurs when the decrease in blood volume is less than 30 per cent. The blood volume is reduced at, or shortly after, injury and remains unchanged for several hours (fig. 13, table 2). After injury the hematocrit values are usually greater (except in splenectomized dogs, fig. 11) than during the control period, but they are maintained at the new levels with little change for several hours (figs. 7 to 11). The plasma protein concentration may increase or decrease slightly. As in the case of the hematocrit reading, the new level remains relatively constant for some time (figs. 7 to 11). The relation of these results to the problem of capillary leakage is discussed.

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# THE PERIPHERAL MOTION ACUITY OF 50 SUBJECTS<sup>1</sup>

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The ability to perceive peripheral motion is recognized to be a primitive retinal function, failing last and returning first in diseased conditions (1, p. 968). The thresholds for perception of peripheral movement, and the many variables operating on them, have been reviewed by Duke-Elder (2, p. 1086-1089). However, the perception of detail within the object perceived to be in motion is a different function. The distinction between simple perception of movement and perception of detail in a moving object must be clearly understood in interpreting this report. The simple perception of movement normally occurs within a very wide range of speed, angular excursion, etc. This wide range makes possible the perception of a moving object in the far periphery when the small size of the object renders it invisible if stationary. Furthermore, under these conditions the movement of the object can be readily seen but the object itself cannot be described in any way (2, p. 1088). Larger stimuli are necessary before the refinements of description known as form perception can be accomplished. This paper deals with the threshold of minimum size for the discernment of simple form in a test object moving across the retinal periphery at constant speed under constant photopic illumination.

**APPARATUS.** A specially constructed perimeter (3) was used. This design facilitated the quick changing and position alteration of Landolt broken circles ranging from the largest with a 10 mm. break to the smallest with a  $\frac{1}{4}$  mm. break. For the study of peripheral acuity with moving test objects a synchronous motor was attached so that it moved the arm of the instrument supporting the test object, rotating it toward or away from the line of central vision along a meridian. A shutter (Ilex no. 4) was suspended in the line of peripheral vision between the test object and the subject's eye. It was supported by the mobile perimeter arm so that it remained in the line of peripheral vision regardless of the angular deviation of the test object or the meridian in which the arm rotated. An electromagnet was mounted below the shutter so that it tripped the shutter mechanism when powered. The electromagnet was wired to a switch mounted so that the rotation of the perimeter arm would turn the power on or off at any desired point of rotation. The switch was adjustable with gauges reading in degrees of angular deviation of the test object from the line of vision, so that exposure of the test object for any desired angular excursion

<sup>1</sup>The apparatus used in these experiments is the property of the Committee on Selection and Training of Aircraft Pilots of the National Research Council. It was constructed for this Committee as part of one of a series of researches conducted under the provisions of a contract between the Civil Aeronautics Authority and the National Research Council, and was lent to the writer for the experiments here reported.

could be accomplished. This insured a variable but absolutely uniform exposure of the test object. With the shutter properly adjusted it could be automatically opened by the electromagnet at any phase of rotation and closed at any phase. The shutter could also be adjusted for time exposures ranging from  $\frac{1}{100}$  second to 1 second. When so adjusted the shutter would open when the electromagnet was powered and close by its own mechanism according to the adjustment. Calibration of the motorized perimeter showed that the test object moved at the rate of  $30^\circ$  in 2.1 to 2.2 seconds, or approximately  $15^\circ$  per second.

The test object was illuminated by a 60 watt Mazda daylight bulb which rotated with the perimeter arm so that the brightness was the same regardless of the angular deviation of the test object. The remainder of the apparatus, targets, test objects, identification button, blindfold, and booth were the same as those used for previous experimentation (4).

**METHODS.** A series of previous researches (4, 5, 6) utilizing 5 meridians revealed identical phenomena in all of them except for the measure of the acuity itself. This varied in keeping with accepted relative values. It was therefore decided that a more exhaustive study of a single meridian, utilizing as many variables as possible, could safely be extended to apply to the remaining meridians, subject only to alteration for different acuity. In this study the horizontal meridian only was tested in both eyes.

The excursion, time of exposure and angular deviation were chosen so as to provide, as nearly as possible, direct comparison with measurements collected with stationary test objects by comparable technique (3, 4, 5). It was decided to compare the acuity measured with inswinging test objects to the acuity measured with outswinging test objects, the conditions being otherwise identical. Preliminary experiments showed that an exposure of the test object for  $15^\circ$  of an arc, amounting to an exposure time of about 1 second, should give adequate results. The following exposures were adopted: (a) in;  $45^\circ$ - $30^\circ$ , (b) out;  $30^\circ$ - $45^\circ$ , (c) in;  $60^\circ$ - $45^\circ$ , (d) out;  $45^\circ$ - $60^\circ$ , (e) in;  $75^\circ$ - $60^\circ$ , and (f) out;  $60^\circ$ - $75^\circ$ . Thus, in the first measure, (a), the shutter opened at  $45^\circ$  and closed at  $30^\circ$  with the test object swinging toward the line of central vision at the rate of  $15^\circ$  per second, etc. Areas (a) and (b) have their near points comparable to the  $30^\circ$  point on the horizontal meridian tested with stationary test objects. Measures (e) and (f) are similarly comparable to the  $60^\circ$  point previously tested on the horizontal meridian with stationary test objects. It soon became evident that areas (e) and (f) showed so poor a motion acuity as to be unmeasurable except in unusual cases, and measures (c) and (d) were therefore adopted. The motion acuity in these latter areas was more amenable to measurement and the measures were therefore retained although not directly comparable to previous stationary acuity data. For the sake of comparison with  $\frac{1}{2}$  second rapid recognition scores made with stationary test objects measures were made with both inswinging and outswinging test objects with the shutter set for  $\frac{1}{2}$  second exposure. These were: in;  $33^\circ$ - $30^\circ$  and out;  $30^\circ$ - $33^\circ$ . The  $\frac{1}{2}$  second shutter adjustment permitted exposure of the test object for approximately a  $3^\circ$  excursion, the switch being set so that the near point of the exposure was at  $30^\circ$

in each case. These measures are comparable with the  $30^\circ$  points similarly tested on the horizontal meridian with stationary test objects (3). The above 8 measures were made on each eye in 50 subjects selected at random, the right eye always being tested first.

The method of determining acuity used in previous experiments with stationary test objects was adopted for the present investigation. The same rigid success criterion was used. The test object was exposed according to the above specifications and the subject was required to signal the position of the circle. The procedure was repeated until the subject had given either 4 consecutive right answers or the second wrong one. The second miss always disqualified. If successful on one size the next smallest test object was used, and so on until failure occurred. The recorded score was the smallest Landolt circle correctly identified (4).

**RESULTS.** In the following discussion the innermost point of exposure of any measure is used to identify it; an excursion of from  $45^\circ$  to  $30^\circ$  or from  $30^\circ$  to  $45^\circ$  is called a  $30^\circ$  measure. Unless the direction of the movement is specified the statements apply to both inswinging and outswinging test objects.

A sharp drop in motion acuity was observed between the  $30^\circ$  measures and the  $60^\circ$  measures, the latter being virtually unobtainable. At the  $30^\circ$  measures there was only one total failure (consisting of failure on the largest test object) in a total of 200 such measures. At  $60^\circ$  there were 144 such failures, or 72 per cent. At  $45^\circ$ , midway between these measures, there were 32 such failures, or 16 per cent.

The means and standard deviations of the motion acuity scores are presented in figure 1. The  $60^\circ$  measures have been omitted because of the meaningless nature of the majority of them. Any assignment of a numerical value to failure on the largest size test object is arbitrary since no actual measure has been obtained. In cases where failure occurred in other measures, notably at  $45^\circ$ , a numerical value was assigned. This was equal to what the score would have been if the subject had been successful on a hypothetical size, no. 11. This hypothetical score is doubtless too small and has the effect of contracting the standard deviation of the recorded scores. This is of importance only in the  $45^\circ$  measures. The same hypothetical value for failure was used in previous experiments whenever this occurred. It will be noted (fig. 1) that there is very little difference between the measures with inswinging test objects and those with outswinging test objects. At  $30^\circ$  in both eyes the inswinging test object rendered slightly weaker acuity than the outswinging test object, the reverse being true in both eyes at  $45^\circ$  and with rapid recognition at  $30^\circ$ . In all cases the difference is less than  $\frac{1}{2}$  sigma and should not be regarded as significant.

A comparison of the acuity obtained at  $30^\circ$  using a  $15^\circ$  excursion of exposure with that obtained at  $30^\circ$  with rapid recognition and its consequent exposure of only  $3^\circ$  reveals very little difference between the two. This indicates that the innermost  $3^\circ$  of the  $15^\circ$  exposure has yielded the essential information. Since the rapid recognition scores are not quite as good as those with long exposure it may be presumed that a very slightly longer exposure, say  $5^\circ$ , would

give identical results when compared with the 15° exposure. Thus it may be said that, when a peripherally perceived object is moving toward or away from the line of vision, the innermost 5° of its excursion is the critical area for perception of its form.

A direct comparison of these acuity scores with those collected with stationary test objects will be helpful. The motion acuity averages 2.8 (in) and 2.5 (out) at 30° with 15° exposure and stationary acuity averages 1.6 (4, fig. 1). The

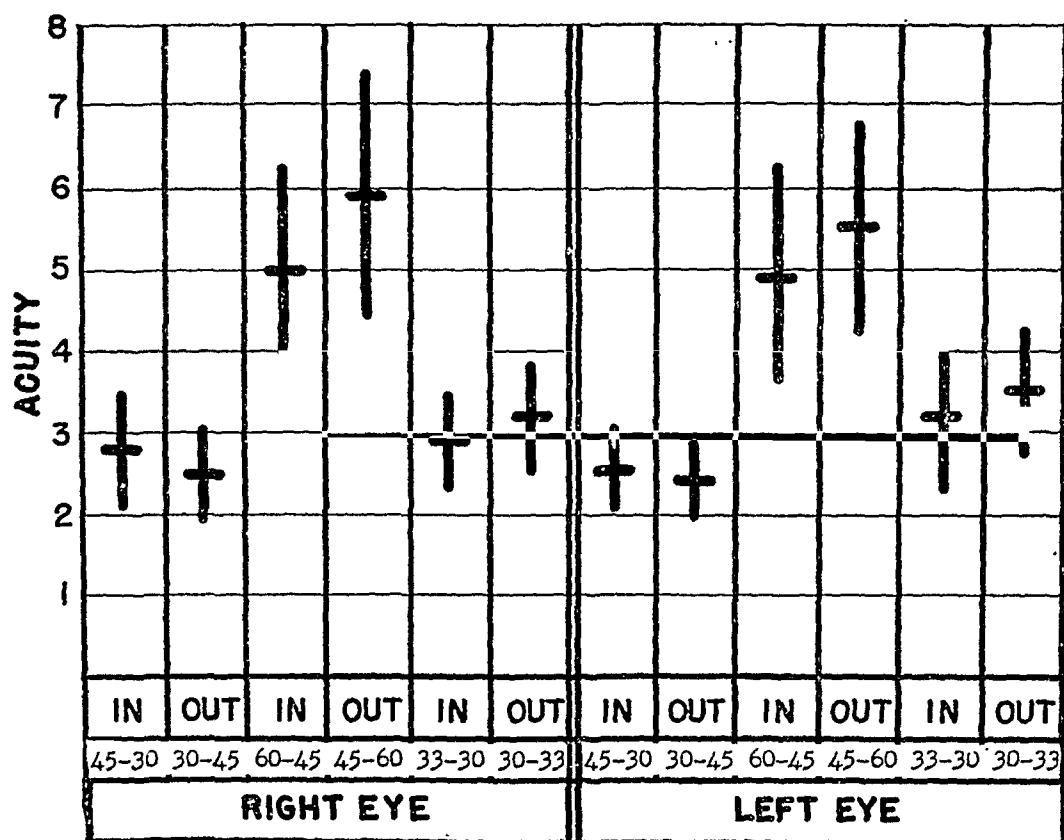


Fig. 1. Graphical representation of means and standard deviations of peripheral motion acuity scores in 50 subjects. Verticals show limits of the standard deviation. Cross bars are means. *In* and *out* refer to the direction of movement. The 4 groups having only 3° exposure were timed at  $\frac{1}{2}$  second.

motion acuity is thus somewhat weaker at 30° than similar acuity with stationary test objects. At 60° stationary acuity averaged nearly 5 (4, fig. 1). A proportionate decrease in motion acuity would bring the 60° measure to somewhat more than 8. However, 72 per cent of the 50 subjects failed this measure, making the average unknown but well over 8. Thus it is evident that the average of the 60° measures for motion acuity is not in the same proportion to the 30° measures as in stationary acuity. It follows that the motion acuity curve drops off rapidly between 30° and 60°. The way in which this occurs is illustrated in figure 2, which shows the frequency distributions of scores at 30°, 45°, and 60° in the left eye with inswinging test objects. At 30° no failures were en-

countered. At  $45^\circ$  the main group shows a decreased acuity but 8 subjects have separated from the normal distribution and entered an unmeasurable group which is clearly separated from the rest. At  $60^\circ$  thirty-two subjects are in the unmeasurable group and only 18 have remained measurable in an irregular distribution. These peculiar distributions indicate a break in the curve for motion acuity as the periphery is approached. In 16 per cent of subjects this occurs between  $30^\circ$  and  $45^\circ$  and separates them from the remaining 84 per cent whose acuity has decreased but whose distribution is normal. Between  $45^\circ$  and  $60^\circ$  another 56 per cent of all subjects have experienced a

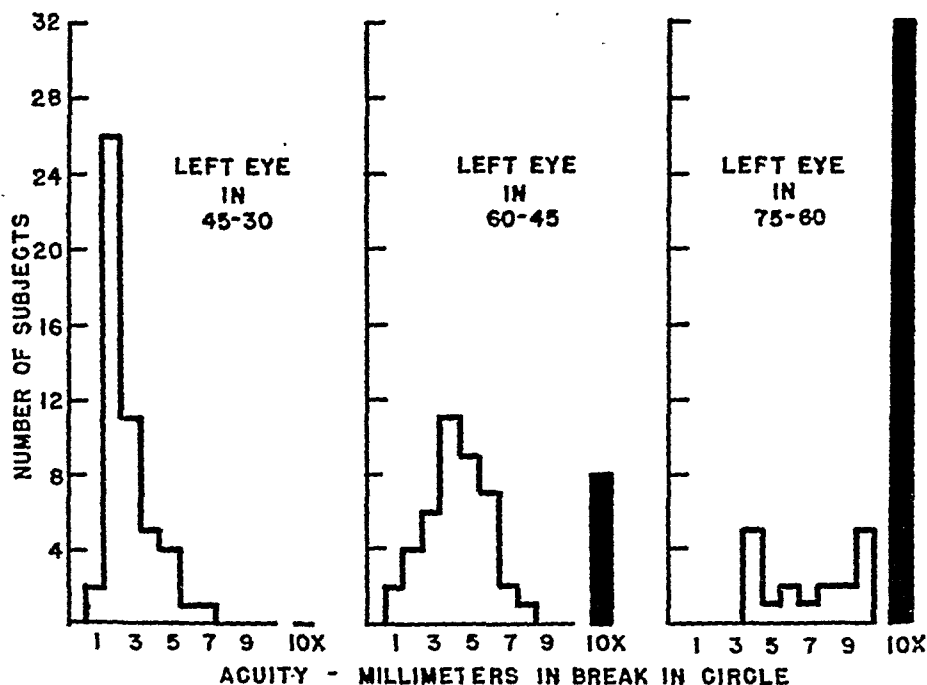


Fig. 2. Frequency distributions of peripheral motion acuity scores measured with inswinging test objects in the left eye of 50 subjects. The excursion of exposure is  $15^\circ$  in all cases. 10X indicates failure on the largest size test object, a Landolt circle with 10 mm. break.

similar loss with 72 per cent now in the unmeasurable group. The above percentages are an average of the measures represented in figure 2 and the three other sets of similar measures (in and out in both eyes) and thus do not exactly correspond with the distributions of figure 2.

The motion acuity scores for rapid recognition at  $30^\circ$  average about 3.2 (fig. 1) with the outswing somewhat weaker than the inswing. In 26 subjects previously tested under conditions of rapid recognition with stationary test objects these same points averaged about 1.9 (3). The discrepancy between these two sets of scores is similar to that between the stationary acuity scores and those collected with moving test objects having a  $15^\circ$  excursion of exposure. This supports the conclusion that simple form acuity in the periphery is weaker for moving objects than for stationary ones, being only 60 per cent as strong.

It was decided to adopt a total score for the present subjects which would

be representative of the subject's over-all ability. A summation of the measures represented in figure 1 was adopted as meeting this requirement. It includes both inswinging and outswinging measures, 8 of which are at  $30^\circ$  and 4 at  $45^\circ$ . The  $60^\circ$  measures have been omitted because of the high proportion of failures. Figure 3 presents a frequency distribution of these total scores. The extremes were 16.3 (best) and 76.5 (worst) with the average falling at 44.4. There is a noticeable tendency toward a binodal distribution. The secondary node, located between 65 and 70, is interpreted to be due to the frequency of total failures in the  $45^\circ$  measures. This has been indicated to have occurred in 16 per cent of cases. Since 4 of the 12 measures are so affected the total scores show a secondary group with disproportionately higher scores than the rest. While the total scores here presented are calculated in the same manner, by summation of individual measures, as the total scores used to represent stationary acuity, the

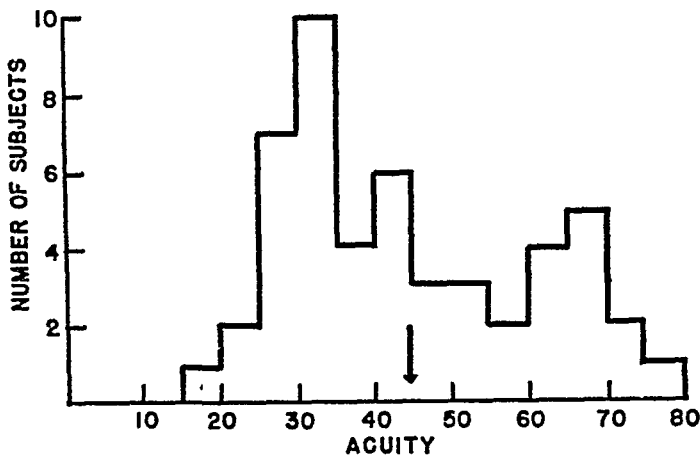


Fig. 3. Frequency distribution of peripheral motion acuity scores in 50 subjects. Arrow indicates mean at 44.4.

two sets are not directly comparable to each other. The stationary acuity scores (5, fig. 2) are derived from ten  $30^\circ$  points and four  $60^\circ$  points on 5 meridians. The motion acuity scores are derived from eight  $30^\circ$  measures by 4 separate techniques and from four  $45^\circ$  measures by 2 techniques on only one meridian.

During the progress of the testing a tendency for the subject's efficiency to fluctuate independently from measure to measure was noticed. This spontaneous fluctuation was noticeable during the testing of stationary acuity and a measure of it has been extracted (6). A similar extraction was made from the present motion acuity scores. It was assumed that the "true" acuity of any point bore a constant relationship to the total score and that the observed fluctuation operated above and below this mean. Therefore a hypothetical score for each measure was calculated from the subject's total score, being based on the relationship of the mean of the score of that measure to the mean total score in 50 subjects. The difference between this hypothetical score and the recorded score for that measure constituted the subject's fluctuation score on that measure. A summation of these scores for all 12 measures is called the "millimeter variability" and is an index of how much the subject wavered spontaneously dur-



ing the measurement of his motion acuity. This score is expressed in millimeters and is directly comparable to the subject's acuity. The millimeter variability scores of the present group is represented in figure 4. These scores ranged from 6.6 (best) to 29.9 (worst) with the average at 12.3.

The relationship of the millimeter variability of the subject to his total score (acuity) is expressed as a percentage by dividing the former by the latter and

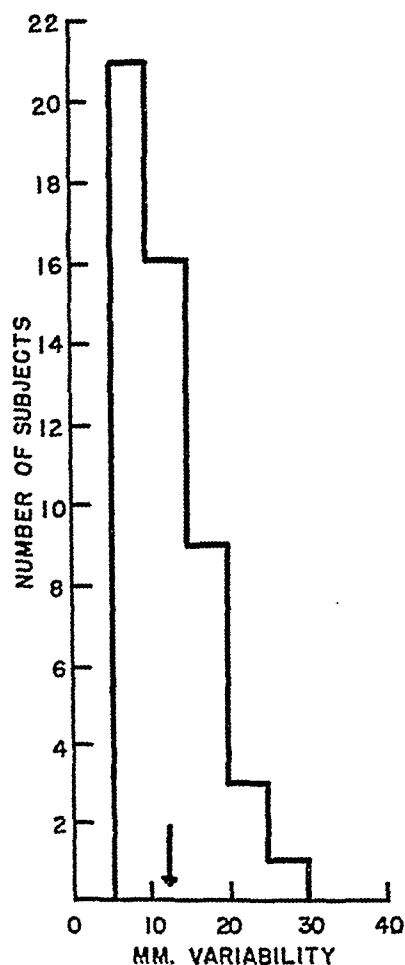


Fig. 4

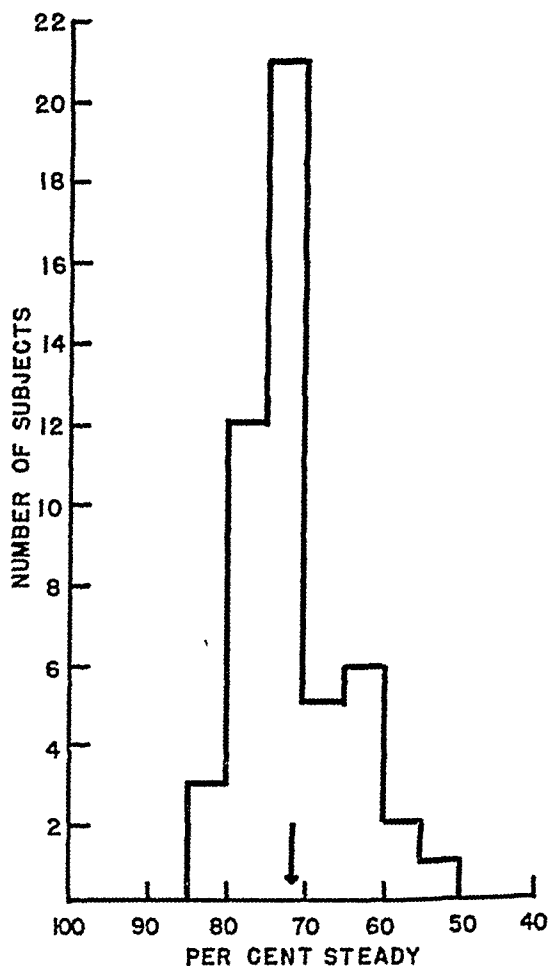


Fig. 5

Fig. 4. Frequency distribution of millimeter variability scores in peripheral motion acuity in 50 subjects. Arrow indicates mean at 12.3.

Fig. 5. Frequency distribution of percentile steadiness scores in peripheral motion acuity in 50 subjects. Arrow indicates mean at 71.9.

multiplying by 100. Subtraction from 100 gives the "percentile steadiness" of the subject. The percentile steadiness scores of the present group are presented in figure 5. They ranged from 82.9 (best) to 53.6 (worst) with the average at 71.9. These percentile steadiness scores, being the relationship of the amount of fluctuation to the over-all ability of the subject, are directly comparable to those derived from stationary acuity scores (6, fig. 2). Stationary photopic acuity was 78 per cent steady and stationary scotopic acuity was 84 per cent steady, both better than the 72 per cent steady observed for motion acuity.

In previous studies of reliability of the various point scores, etc., the Pearson product-moment method of correlation was used (6). Similar calculations were made on the present group. An eye to eye correlation of the total scores for each eye rendered a coefficient of 0.70 which, after correction by the Spearman-Brown formula, became 0.82. This compares with a reliability of 0.91 for photopic stationary scores (4) and 0.84 for scotopic stationary scores (5). Correlations of individual scores of each measure in our present group with the total scores yielded coefficients ranging from 0.57 to 0.83 with overlap of the non-significant range of variation in all cases. This compares to ranges of 0.60 to 0.77 for photopic stationary acuity and 0.44 to 0.78 for scotopic stationary acuity. No starting difficulty, as observed in scotopic stationary acuity (5), was observed since the first measure yielded a coefficient of 0.70. The scores for all of the measures on which the test object swung inward were converted by summation to a total score and this was correlated with a similarly calculated total score derived from all measures collected with outswinging test objects. The coefficient was 0.86 and serves to illustrate the close relationship of measures obtained from inswinging test objects with those obtained from outswinging test objects. The percentile steadiness scores did not show a significant correlation with the acuity scores. An eye to eye correlation of the millimeter variability scores was not significant, reaffirming previous conceptions of its inherently changeable character (6, 581).

A significant feature of previous studies was the tendency toward improvement through practice which was made the subject of a special study (3). This was evident in numerous ways, not the least of which was an eye to eye improvement during tests regardless of which eye was tested first. Such improvement is hardly noticeable in the present motion acuity scores. The average score of the first eye tested (right) was 22.3 and the average score for the second eye (left) was 22.0. This slight improvement of 1.6 per cent is considerably less than that observed in stationary test scores. In interpreting this lack of improvement it is significant to realize that the technique imposes what is, in effect, the requirement of rapid recognition of the test object. Although the test object has been presented for an excursion of  $15^\circ$  amounting to an exposure of one second in 8 out of 12 measures, it has been shown that the critical exposure occurs in the innermost  $5^\circ$ . This results in a useful exposure of  $\frac{1}{3}$  second or less. A similar lack of improvement has been observed in the scores of 26 subjects whose stationary acuity was measured with only  $\frac{1}{3}$  second exposure (3). In this group the score of the first eye was 35.5 and the second, 36.2, a slight decline. It is considered that, in respect to improvement, the motion acuity scores are comparable to the rapid recognition scores for reasons of brief exposure of the test object.

DISCUSSION. Although the perception of peripheral motion as such is known to be well developed, the experiments here reported indicate that the ability to perceive form in a moving object is measurably less than form perception for stationary objects. Also, this ability becomes so poor as to be negligible between  $30^\circ$  and  $60^\circ$  in most individuals. The measures were all made with the test object moving directly toward or away from the line of central vision

along a meridian and thus traversed areas of greatly differing acuity. This circumstance was reflected in the clear evidence establishing the innermost  $5^{\circ}$  as the critical area of exposure. The outer  $10^{\circ}$  of exposure traversed retinal areas of such poor relative acuity that this portion of the exposure was useless for form perception.

It is tempting to interpret the poorer acuity observed with moving test objects to be due to the brief exposure of the stimulus. But such an interpretation is not supported by the relative stationary and motion acuity scores with  $\frac{1}{2}$  second exposure each. Here the stationary acuity scores are better than those for motion acuity. They are better in about the same proportion as the stationary acuity scores with unlimited exposure time are better than the motion acuity scores with  $15^{\circ}$  exposure. Thus it must be concluded that motion acuity is weaker than stationary acuity because of the motion itself rather than because of any limitation in the time of useful exposure imposed by the technique. It may be argued that the transit of the image of the test object across the retinal mosaic, which is the fundamental characteristic of a moving image, imposes by its inherent nature a limited time of exposure of the stimulus on any retinal area. In this sense it may be true that the poor form acuity observed for moving objects is due to restricted exposure time. Our present data are not sufficient to make this point clear, but it is possible that the decrease in form acuity with moving test objects may be a function of the speed of the movement. At present, conclusions must be limited to the statement that, given equal time of exposure for the entire stimulus, the simple form acuity is weaker if the object is in motion.

The failure of motion acuity to improve from one eye to the other, contrary to observations of stationary acuity, should not be interpreted to mean that motion acuity cannot be improved. During the stationary acuity tests the subjects were given unlimited time to give their interpretation of test object position. This was utilized by most to such an extent that these tests took much longer to give than either the rapid recognition or the motion acuity tests. The subjective phenomena encountered during the testing of stationary acuity were dominated by the appearance of clear resolutions upon attentive waiting (6, p. 577). The absence of eye to eye improvement in both rapid recognition and motion acuity tests suggests that, in addition to the forcing of peripheral evaluation by controlled fixation (6, p. 579), the improvement of peripheral acuity by practice requires unlimited time of stimulus exposure. It is believed that the failure to improve from eye to eye in the motion acuity test is due to the limited time for which the stimulus was exposed. The subjects did not have adequate time to "work out" an impression of the form of the test objects. Any attempt to train peripheral acuity by the use of moving test objects which simulate actual field conditions would arrest rather than promote the desired improvement. However, improvement in peripheral acuity acquired through a course of training with stationary test objects probably transfers to motion acuity. Strong evidence in favor of this supposition has been presented (6, p. 580) in the form of greatly increased awareness of peripheral visual stimuli in everyday life following a course of training. Under these practical circum-

stances peripheral stimuli are almost constantly in motion. Further supporting evidence of successful transfer is found in rapid recognition scores collected with stationary test objects both before and after training stationary acuity with unlimited time of exposure. It has already been mentioned that eye to eye improvement was absent in these subjects. Nevertheless, after training with stationary test objects with unlimited time of exposure, it was found that the rapid recognition scores had changed from 76.7 to 41.3, an improvement of 85 per cent. It seems reasonable to conclude that improvement of peripheral acuity resulting from systematic practice with long-exposed stationary test objects transfers successfully to motion acuity although improvement through practice with moving test objects is not readily accomplished.

The correlations of motion acuity scores stress the resemblance of peripheral motion acuity to stationary acuity. Further evidence of similarity is found in the millimeter variability and percentile steadiness scores of the two groups. In general it may be said that the same basic phenomena have been observed in the perception of form in moving test objects in the periphery as in the perception of form in stationary ones. The distinguishing features are (a) an inherently weaker acuity with moving objects, (b) a rapid falling off of the motion acuity curves between  $30^{\circ}$  and  $60^{\circ}$  in 72 per cent of subjects, and (c) little or no tendency for motion acuity to improve through practice.

#### SUMMARY

1. Simple form acuity in the retinal periphery was tested with moving test objects in 50 subjects selected at random.
2. The peripheral motion acuity test showed a reliability of 0.82.
3. Peripheral motion acuity is inherently weaker than peripheral acuity measured with stationary test objects, being only 60 per cent as strong.
4. Peripheral motion acuity shows little or no tendency to improve through practice, probably because of restriction of exposure time.
5. Peripheral motion acuity measured with test objects moving toward the line of central vision correlates with motion acuity measured with test objects moving away from the line of central vision with a coefficient of 0.86.
6. Peripheral motion acuity curves fall off rapidly between  $30^{\circ}$  and  $60^{\circ}$  from the line of vision in 72 per cent of cases.
7. Peripheral motion acuity is 72 per cent steady.
8. The phenomena observed during the testing of peripheral motion acuity are essentially the same as those observed during testing with stationary stimuli.

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# THE EFFECT OF SUBCUTANEOUS HISTAMINE INJECTION ON THE CARDIAC OUTPUT OF THE UNANESTHETIZED DOG<sup>1</sup>

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Histamine poisoning has been considered as a possible factor in the development of anaphylactic and traumatic shock since the early studies of Dale and his co-workers (5, 6). In a comparison between histamine poisoning and traumatic shock (7), it was found that the subcutaneous injection of 10 mgm. of histamine per kilogram produced in unanesthetized dogs a syndrome characterized by marked hypotension and tachycardia, but without the reduction of blood volume and with little of the central nervous depression characteristic of traumatic shock. Since disturbances of circulation affect the mammalian organism in more or less direct relation to the degree to which they interfere with the blood flow to the tissues, it seemed of interest to study the changes in cardiac output and oxygen consumption produced by subcutaneous histamine injection.

**METHODS.** Experiments were carried out on 7 normal dogs ranging in weight from 6.5 to 9.2 kgm. Under local anesthesia (2 per cent procaine), the trachea was cannulated and one jugular vein, femoral vein and femoral artery were exposed. After a control period of one or more hours, approximately 10 mgm. of histamine base per kilogram of body weight were injected subcutaneously in the form of histamine diphosphate dissolved in 0.9 per cent NaCl.

Mean blood pressure measurements (mercury manometer) were obtained by direct arterial puncture, and heart rates were counted with a stethoscope. O<sub>2</sub> consumption and apparent R.Q. were measured continuously during the control period and for some five hours after histamine injection, using a Scholander respirometer (13). At intervals before and after histamine injection, approximately simultaneous femoral arterial and mixed venous blood samples were drawn into heparinized syringes. Mixed venous blood was obtained from the right heart by means of a catheter inserted through the left external jugular vein. In 5 experiments additional samples were taken from the right external jugular vein. Blood samples were analyzed for O<sub>2</sub> content using the method of Van Slyke and Neill (16). Hematocrit values of femoral arterial blood were obtained after centrifugation in Wintrobe tubes at 3000 r.p.m. for 30 minutes.

Cardiac output was calculated from the ratio of O<sub>2</sub> consumption to the arterial-

<sup>1</sup> This work was done partly under a grant from the Josiah Macy, Jr. Foundation, and partly under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the College of Physicians and Surgeons, Columbia University.

mixed venous blood  $O_2$  difference (Fick principle). The average stroke volume was estimated from the ratio of cardiac output to heart rate. In addition, total peripheral resistance (T.P.R.) in absolute units (A.U.) was computed from the formula (1):

$$\text{T.P.R.} = \frac{\text{mean arterial pressure} \times 1332}{\text{Cardiac output per second}}$$

A discussion of some of the sources of error in these measurements, as carried out in this laboratory, will be published elsewhere (11).

**RESULTS.** The effects of histamine injection on various circulatory and respiratory measurements carried out are shown in table 1. Figure 1 illustrates a typical experiment. It may be seen in table 1 that the control  $O_2$  consumption of the 7 animals ranged from 70 to 138 cc. per minute. It varied from 49 to 79 per cent of the control value in the first half hour after histamine injection, and in most of the experiments subsequently increased. One dog (expt. 13), which died 5 hours after histamine, showed a progressive fall in  $O_2$  consumption throughout the experiment. Usually the apparent R.Q. rose immediately after histamine, returning toward the control level in the course of  $\frac{1}{2}$  to 4 hours.

The arterial  $O_2$  content increased after histamine injection, closely paralleling the rise in hematocrit value. The A-V  $O_2$  difference, which ranged from 2.0 to 8.3 vols. per cent before histamine injection, rose sharply in the first half hour after histamine, varying at this time between 9.4 and 16.5 vols. per cent. The highest A-V  $O_2$  difference shown in any experiment was 18.0 vols. per cent. This was seen in dog 13, 2 hours after histamine injection and 3 hours before death. In general, the A-V  $O_2$  difference decreased between the second and fifth hours following the injections, but in no instance did it return to the control level within 5 hours.

The reduction of  $O_2$  consumption and the appreciable rise of A-V  $O_2$  difference were accompanied by a marked fall in cardiac output (table 1 and fig. 1). The control cardiac outputs varied between 1.16 and 5.85 L. per minute, probably indicating that the animals were not all in a basal state during the control determinations. One-quarter to one-half hour after histamine injection, 6 of the 7 dogs had cardiac outputs lying between 0.40 and 0.47 L. per minute. Only one dog (expt. 11) showed a cardiac output (0.76 L. per minute) outside of this narrow range. With one exception (dog 13) the cardiac output increased as the blood pressure gradually rose. Since the heart rate always increased after histamine injection, the stroke volume was reduced relatively more than the cardiac output. Thus, the stroke volume which in the control determinations varied from 11.4 to 45.4 cc. ranged between 2.5 and 5.2 cc. shortly after injection and in no case rose above 5.3 cc. in the subsequent 3 to 5 hours.

The mean arterial pressure measured at the time of the cardiac output determinations remained below 50 mm. Hg throughout most of the experimental period. The T.P.R. increased significantly and remained elevated after histamine in 5 of the 7 experiments. Dogs 5 and 11, however, showed a decrease in T.P.R.

TABLE 1

*The effect of subcutaneous histamine upon respiration and circulation of the unanesthetized dog*

DOG NO.	BODY WEIGHT	TIME AFTER HISTAMINE	OXYGEN CON-SUMP-TION	R.Q.	BLOOD OXYGEN CONTENT			ARTERIAL HEMATO-CRIT	CARDIAC OUTPUT		T.P.R.
					Vols. %				l./min.	cc./beat	
					Femoral arterial	Mixed venous	Jugular venous				
			cc./min.					%			A.U.
5	7.6	Control	85	0.76	17.2	9.9		48.0	1.16	11.4	8300
		15 min.	63	0.87	19.5	6.0		54.0	0.47	3.1	6000
		2 hr. 15 min.	95	0.74	22.2	12.1		56.3	0.94	4.5	3800
6	8.7	Control	100	0.86	20.3	15.9	12.8	54.0	2.27	22.4	4800
		26 min.	67	0.77	22.9	7.2	7.1	62.8	0.43	3.4	7800
		2 hr. 38 min.	84	0.82	23.3	12.0	15.4	64.5	0.74	4.2	5400
		3 hr. 30 min.	89	0.83	24.1	9.4	13.1	64.0	0.60	3.1	6300
7*	9.2	Control	117	0.79	19.2	17.2	16.2	50.5	5.85	45.4	1900
		28 min.	66	0.85	20.8	6.7	9.5	53.2	0.47	3.5	5500
		1 hr. 38 min.	71	0.84	21.0	8.0	15.7	55.2	0.55	3.8	4100
		4 hr. 45 min.	81	0.75	24.6	14.8	15.4	60.6	0.83	5.3	6000
8†	7.9	Control	138	0.77	17.6	13.7	13.0	47.4	3.54	32.2	2700
		28 min.	67	0.85	21.5	5.0		54.5	0.41	2.6	6100
		2 hr. 10 min.	77	0.79	20.8	13.6	16.5	56.1	1.07	5.1	3500
		4 hr. 12 min.	81	0.78	25.1	12.4	18.9	59.6	0.64	3.2	7100
11‡	7.1	Control	92	0.74	11.4	7.5	7.5	32.0	2.36	18.0	4100
		39 min.	71	0.95	13.2	3.8	3.9	38.8	0.76	5.2	3400
		1 hr. 47 min.	82	0.88	15.8	4.5	4.9	41.0	0.72	4.9	3900
		3 hr. 40 min.	60	0.82	16.1	2.8	2.5	44.8	0.45	2.7	4400
12	6.5	Control	70	0.82	16.4	14.3	12.0	48.0	3.34	37.1	2900
		34 min.	51	0.96	18.7	6.9	7.7	50.4	0.43	2.8	6500
		2 hr. 37 min.	61	0.87	20.6	11.7	12.5	53.3	0.68	3.4	5200
13‡	7.5	Control	90	0.74	16.3	13.0		48.4	2.72	28.6	3600
		30 min.	66	0.95	20.9	4.4		57.0	0.40	2.5	5800
		1 hr. 56 min.	58	0.84	21.6	3.6		60.5	0.32	1.5	9300
		3 hr. 35 min.	54	0.92	22.0	6.3		65.2	0.34	1.7	8000

\* Pregnant dog.

† At autopsy the tip of the venous catheter was found to be in the vena cava 1.5 cm. below its opening into the atrium.

‡ Dogs died 4 to 5 hours after histamine.

$$\text{Heart rate} = \frac{\text{cardiac output}}{\text{output per beat}}$$

$$\text{Mean blood pressure} = \frac{\text{T.P.R. (cardiac output/second)}}{1332}$$

Plasma and blood volume measurements made in these experiments have been published elsewhere. For reference purposes the above animals bear the same numbers as in the previous publication (7).

DISCUSSION. Marked reduction of cardiac output after intravenous or subcutaneous histamine injection in dogs has been described previously (3, 8). Johnson and Blalock (10), however, found no change or an increase of cardiac output of barbitalized dogs following subcutaneous histamine injection, but the doses of histamine used were relatively small. Striking pathological changes of the myocardium have been described after subcutaneous histamine injection in unanesthetized dogs (9, 15), and it has been suggested that the primary effect of

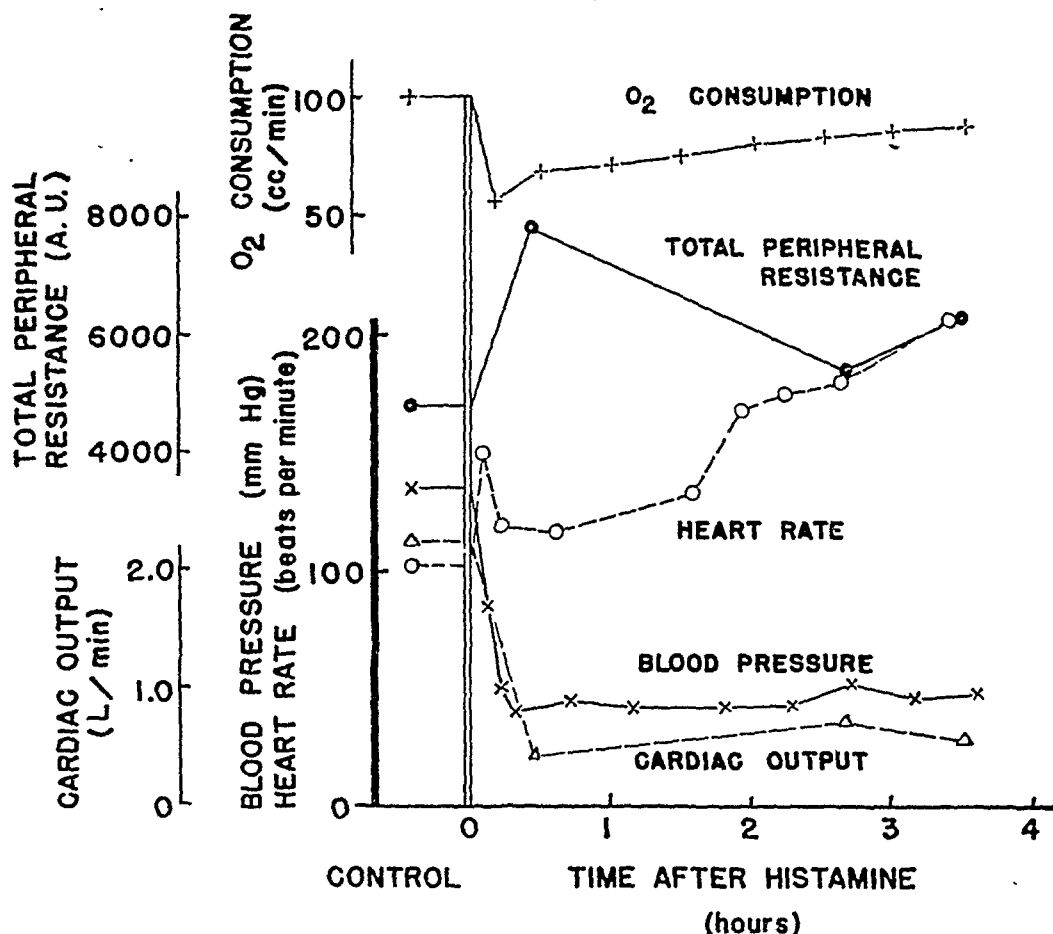


Fig. 1. The effect of subcutaneous histamine injection on the cardiac output, mean arterial blood pressure, heart rate, total peripheral resistance, and oxygen consumption of dog 6.

histamine in the dog may be a specific toxic action on the heart (12 and others). Nevertheless, the importance of cardiac damage in the production of histamine hypotension has not been established.

Other work done in this laboratory (11) shows that in hemorrhagic and traumatic shock in unanesthetized dogs, cardiac outputs of the same order of magnitude as those found in the present series of experiments are associated with higher levels of mean arterial pressure. These results imply that in addition to the decrease in cardiac output some other factor must be considered in explaining the extremely low arterial pressure of histamine poisoning. The observation



that histamine produces arteriolar dilatation in the dog (4) suggests that histamine hypotension may be the result of vasodilatation as well as reduction in cardiac output. If this is true, the increase in total peripheral resistance which occurred in most of our experiments (table 1) probably results from an increase in blood viscosity caused by the rise in red cell concentration (14, 17).

In our experiments the decrease in mixed venous  $O_2$  content is related to the slowed blood flow through, and the lowered  $O_2$  consumption of the tissues (fig. 1). Since it has been shown that after hemorrhage or trauma there occurs a generalized decrease in the  $O_2$  content of the blood draining various regions of the body (2), we have compared the  $O_2$  contents of the jugular and mixed venous bloods (table 1). These values show a general linear relation before and after histamine injection indicating that under these conditions jugular venous  $O_2$  content can be used as a rough index of cardiac output. This is in agreement with the data obtained in other studies (2, 11).

#### SUMMARY

The subcutaneous injection of about 10 mgm. histamine base per kilogram produced in unanesthetized dogs a marked reduction in cardiac output, A-V  $O_2$  difference and  $O_2$  consumption (table 1, fig. 1). In most of the experiments total peripheral resistance was increased in spite of the profound state of hypotension.

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# THE EFFECT OF BLOOD CARBOXYHEMOGLOBIN CONCENTRATION ON HYPOXIA TOLERANCE<sup>1</sup>

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Carbon monoxide poisoning as a clinical phenomenon has been studied quite extensively. Blood carboxyhemoglobin (COHb) concentrations below 20 per cent, however, received little attention prior to the recent stimulus of military aviation because such concentrations produce no symptoms at sea level. Since CO poisoning may be regarded as comparable to altitude anoxia, it follows that the effects of CO poisoning and of altitude should be additive and predictable. It has been shown (1) that the CO concentrations which obtain in some aircraft may lead to concentrations of at least 12 per cent COHb in the pilot's blood under operating conditions. Consequently, for military reasons a study was initiated on the effects of such blood COHb concentrations at intermediate altitudes where airborne personnel do not ordinarily use oxygen breathing equipment but, nevertheless, suffer reduced arterial oxygen saturations.

A prerequisite of this study is a simple and effective criterion of hypoxia tolerance. The present authors were led to the use of pulse rate during and following exercise as such a criterion by the suggestive data of Major Hingston published by Barcroft (2). His data on one individual showed no effect on the sitting pulse rate up to 16,500 feet, above which a sharp break occurred. The standing pulse rate showed a gentle rise up to 16,500 feet, above which a similar break occurred. The pulse rate after "regulated exercise," however, showed a steep, almost linear rise from sea level to 21,000 feet. This last measurement appeared to be the most sensitive to altitude changes.

**METHODS.** Ten seamen, 17 to 33 years of age, were used as subjects. All subjects stayed in quarters on the nights preceding experimental days, had eight or more hours of sleep between 2200 and 0700, three meals a day in the standard Navy mess, and no alcohol intake.

Two levels of increment ( $\Delta$ ) in blood COHb were used, i.e., 6 per cent and 13 per cent. Preliminary experiments indicated that the normal concentration of COHb in the blood of the subjects seldom varied by more than 1.5 per cent on successive days during the period of the experiment. Hence it was considered unnecessary to restrict or control smoking habits. Knowing the normal level of blood COHb in each subject, in order to obtain the desired per cent  $\Delta$  COHb it was only necessary to apply the following formula which was derived and applied by Pace et al. (3).

$$\text{Per cent } \Delta \text{ COHb} = \frac{\text{Parts CO}/10,000 \times \text{Min. Vol. (STP)} \times \text{exposure time}}{46.5 \times (3.0 \times \text{m.}^2 \text{ body surface area})}$$

<sup>1</sup> The opinions or conclusions contained in this paper are those of the authors. They do not necessarily reflect the views or endorsement of the Navy Department.

The expression ( $3.0 \times m.^2$  body surface area) is a means of estimating total blood volume (4).

Two concentrations of CO were used: 30 parts per 10,000 to obtain 6 per cent  $\Delta$  COHb, and 60 parts per 10,000 to obtain 13 per cent  $\Delta$  COHb. Total volume of gas breathed was controlled, rather than minute volume and time, and the volume given varied with the blood volume of the individual. The CO-air mixture was administered to each subject immediately before his test through a face mask leading from a Tissot gasometer. The blood CO content was determined by the method of Scholander and Roughton (5) on finger bloods taken according to the method of those authors.

In sea level experiments the subjects inspired room air. The three altitudes of 7,000, 10,000 and 15,500 feet were simulated with mixtures of 16, 14 and 11 per cent respectively of oxygen in nitrogen, which were inspired through a demand system at the prevailing barometric pressure. These mixtures were accurate to within  $\pm 0.1$  per cent oxygen. Low oxygen mixtures were used in preference to reduced barometric pressure for inducing hypoxia, because it was desired to administer a rigidly controlled exercise and this was difficult to do in an altitude chamber. It will be shown under Results that the use of oxygen-nitrogen mixtures is justified on the basis of arterial oxygen saturation.

All ten subjects were used each day that a hypoxia tolerance test was made, and each subject was used at the same hour,  $\pm 30$  minutes, in order to eliminate the possible effects of a diurnal rhythm. The subjects were thoroughly indoctrinated in treadmill walking. The test procedure was as follows. A finger blood sample was taken on the subject to determine his normal level of COHb. He then breathes an amount of CO-air mixture which was predetermined on the basis of the percent  $\Delta$  COHb desired and his blood volume. Another finger blood was taken just before the test. Each test lasted 25 minutes, during which time the subject continuously breathed the desired oxygen-nitrogen mixture through a mouth piece. The test was subdivided into three periods: (A) a ten minute "rest period" during which the subject stood erect on the motionless treadmill; (B) a five minute "exercise period" during which he walked on the treadmill which was motor driven at 3 mph on a 2 degree grade; (C) a ten minute recovery period. This latter was subdivided into two five minute periods termed "recovery I" and "recovery II" in order to separate the more rapid phase of recovery from the slower phase following it. A final finger blood was taken immediately after the end of recovery II, and this terminated the test.

Throughout the 25 minutes of the test 30-second pulse counts were made every minute by palpation of the brachial artery, 30-second counts of respiration rate were made every minute, and rate of ventilation was determined by collecting the expired air in a Tissot gasometer, which was read every minute. On days when no CO was administered the oxygen saturation of arterial blood was determined each minute by the oximeter. In all experiments the treadmill room was maintained at a dry bulb temperature of 80° F. and at a relative humidity of 50 per cent.

RESULTS. The summarized data for all tests are presented in table 1; each

TABLE 1  
Summary of the mean values of the various physiological measures during the different periods of the tests

DATE	O <sub>2</sub> BREATHED %	ALTITUDE SIMULATED  feet	OXIMETER READINGS (% SATURATION)				COHb CONCENTRATION				PULSE RATE (BEATS/MIN.)				RESPIRATORY RATE (PER MIN.)				MINUTE VOLUME (LITERS)			
			Rest	Exercise	Recovery I	Recovery II	Normal	Before	After	Mean	Rest	Exercise	Recovery I	Recovery II	Rest	Exercise	Recovery I	Recovery II	Rest	Exercise	Recovery I	Recovery II
Sep. 11.....	20.9	Sea level	95.2	94.7	93.6	92.6	2.66*			Δ %	85.4	103.2	88.8	85.0	17.2	22.4	18.7	18.0	9.4	19.8	11.7	9.7
Sep. 15.....	20.9	Sea level	96.1	95.2	93.3	91.9	3.52	7.04	5.56	6.30	89.9	105.9	93.2	90.9	16.8	24.9	19.5	18.3	10.1	21.3	12.8	10.5
Sep. 12.....	20.9	Sea level					2.88*	14.21	11.70	12.95	85.0	103.8	89.7	85.5	17.3	23.5	18.2	17.4	9.9	20.0	11.6	9.9
Sep. 14.....	20.9	Sea level									92.1	111.9	97.7	93.4	17.7	24.0	18.3	16.8	10.2	20.8	11.5	9.6
Sep. 23.....	16	7,000	90.8	86.4	88.1	86.4	3.14				88.0	106.2	93.4	89.7	16.3	19.7	17.8	17.0	8.7	18.2	10.8	9.0
Oct. 1.....	16	7,000	93.9	91.3	91.4	90.2	2.90	7.27	6.02	6.64	91.9	112.5	99.1	93.7	17.5	20.6	18.2	17.2	10.0	20.0	11.8	9.6
Sep. 25.....	16	7,000						14.95	(12.69)	(13.82)	94.9	112.1	100.1	96.5	16.8	20.1	17.8	17.0	10.6	19.9	12.2	9.8
Sep. 26.....	16	7,000					3.26				97.4	115.4	103.5	97.8	17.9	20.7	18.4	17.6	10.4	20.0	11.8	9.9
Sep. 24.....	14	10,000	88.2	81.5	84.4	83.1					97.0	113.1	103.6	99.4	16.9	20.4	17.5	15.9	9.6	20.9	11.7	9.6
Oct. 3.....	14	10,000	88.5	82.8	83.7	82.5	3.39				95.0	117.9	102.7	96.2	18.5	22.0	18.7	18.9	10.0	21.0	11.2	9.3
Sep. 29.....	14	10,000					3.64	6.10	(4.74)	(5.42)	96.5	118.9	103.5	98.0	18.5	21.7	18.4	17.4	11.1	21.4	12.0	10.3
Sep. 28.....	14	10,000						14.44	12.40	13.42	99.1	122.5	109.3	103.2	17.5	20.2	17.7	16.6	10.4	21.0	12.2	10.6
Sep. 27.....	11	15,500	83.8	73.8	75.9	75.7	3.12				96.6	123.3	108.9	100.1	16.6	21.3	17.2	17.1	10.1	23.7	13.5	10.3
Sep. 30.....	11	15,500					3.45	6.16	(4.80)	(5.48)	102.3	130.9	116.7	107.3	17.5	21.4	18.0	17.7	12.4	25.7	12.0	10.5
Oct. 2.....	11	15,500						12.62	10.89	11.75	102.9	132.1	118.5	108.6	17.7	22.2	19.2	17.5	11.8	25.4	13.7	10.5

\* On these two dates 5 of the 10 subjects were not allowed to smoke. Figures in parentheses are explained under Results.

figure is the mean for all ten subjects for the period indicated. The rest period values are 10 minute means, while values for the exercise period and the two recovery periods are means of five minutes. A general appraisal of all the values for pulse rate, respiratory rate, minute volume, and tidal volume indicates that the pulse rate during exercise is the measurement which is most sensitive to changes in both altitude and percent COHb. Moreover, the pulse rate during rest and recovery proved more sensitive to these changes than did any of the respiratory measures. The respiratory rate, minute volume, and tidal volume during exercise tend to increase slightly with increasing altitude, but show no response to variations in blood COHb within the range studied. This corroborates the findings of Asmussen and Chiodi (6). Since the respiratory measures throw little light on the problem involved, they are not considered further.

It will be noted that some of the data on blood COHb in table 2 are enclosed in parentheses. When three blood samples were taken on each man, the total number of samples to be analyzed was thirty, and at least two analyses had to be done on each. In order to lighten the analytic load, bloods after the recovery period were omitted on three experimental days. This was considered justifiable since, as may be seen in table 1, the fall in percent COHb during the course of a test was quite constant at each level of COHb concentration. Consequently, on the three days in question the percent  $\Delta$  COHb "after" and the mean per cent  $\Delta$  COHb were derived by subtracting an average value for the fall in per cent COHb during comparable experiments from the  $\Delta$  COHb "before". All values involving such assumptions are enclosed in parentheses.

The pulse rate data from two typical test days are presented in figure 1. Each point on the curves is the minute mean for 10 subjects. The two curves presented represent the extreme experimental conditions; that at sea level with 0 per cent  $\Delta$  COHb placing the least stress on the subjects, and that at a simulated altitude of 15,500 feet with 13 per cent  $\Delta$  COHb imposing the greatest stress. On the curve for 15,500 feet one standard deviation on either side of the mean is indicated by the broken lines in order to give some indication of the variability of the individual values. The standard deviation is roughly 10 beats per minute, and the standard error of the means is approximately 3 beats per minute. The variability of all tests was of this magnitude.

The pulse rate curves for all the other series of tests fall at well-defined levels between these two curves and are closely parallel to them. It may be noted from figure 1 that the pulse rate range covered is narrowest in the rest period and widest in the exercise period. This is another indication that the exercise pulse rates show the greatest sensitivity to the experimental variables.

Figure 2 presents data obtained at an altitude of 7,000 feet. It illustrates the range of mean values that is obtained by varying the per cent COHb alone. The means of 0 per cent  $\Delta$  COHb and 13 per cent  $\Delta$  are separated in this case by 12 to 15 beats per minute during the exercise period.

Figures 3 and 4 showing pulse rate plotted against altitude hold the most significance for the present study. Each point represents the mean of the five minute exercise period (fig. 3) or of the recovery I period (fig. 4) for all subjects.

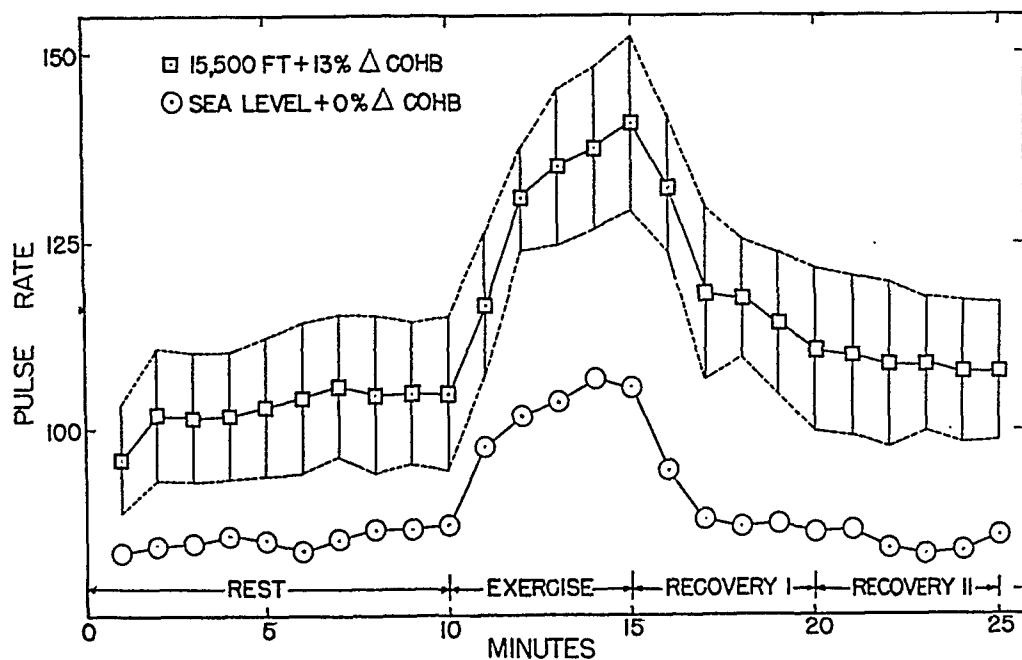


Fig. 1. Pulse rate changes obtained in individual test runs. The points are minute averages for 10 subjects. The two curves define the range covered by all test means. The broken lines indicate  $\pm 1$  standard deviation. For the sake of clarity standard deviations were omitted on the sea level curve, since it was approximately the same on all tests. The standard error of the mean values plotted is  $\pm 3$  beats per min.

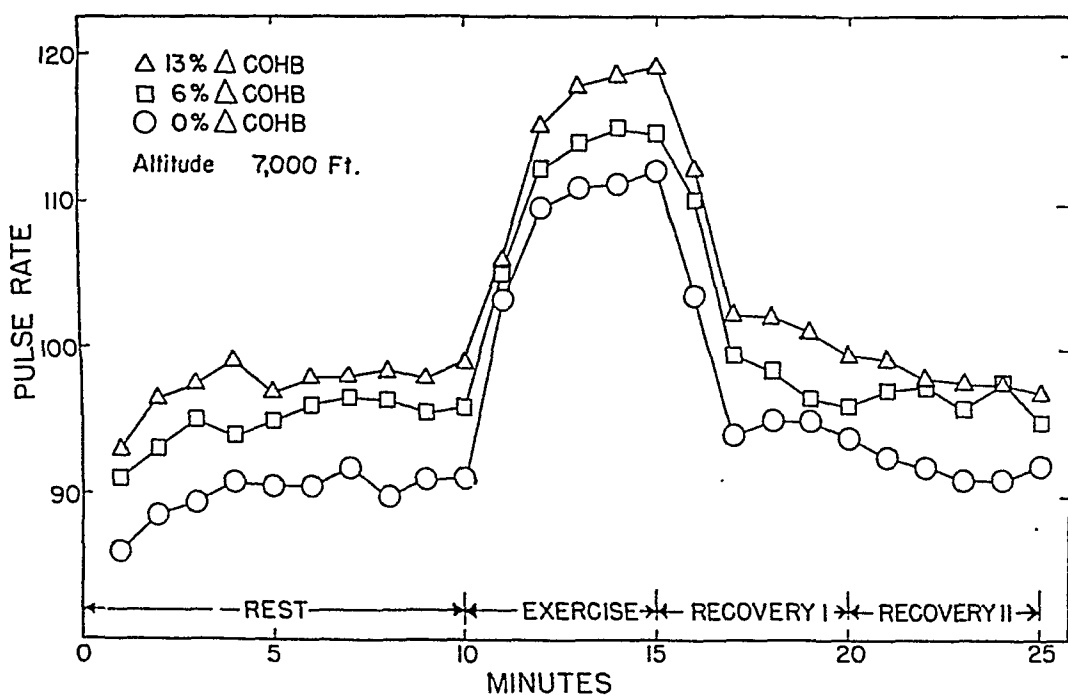


Fig. 2. Effect of COHb on pulse rate at constant altitude. Each point is a minute mean value for 10 subjects.

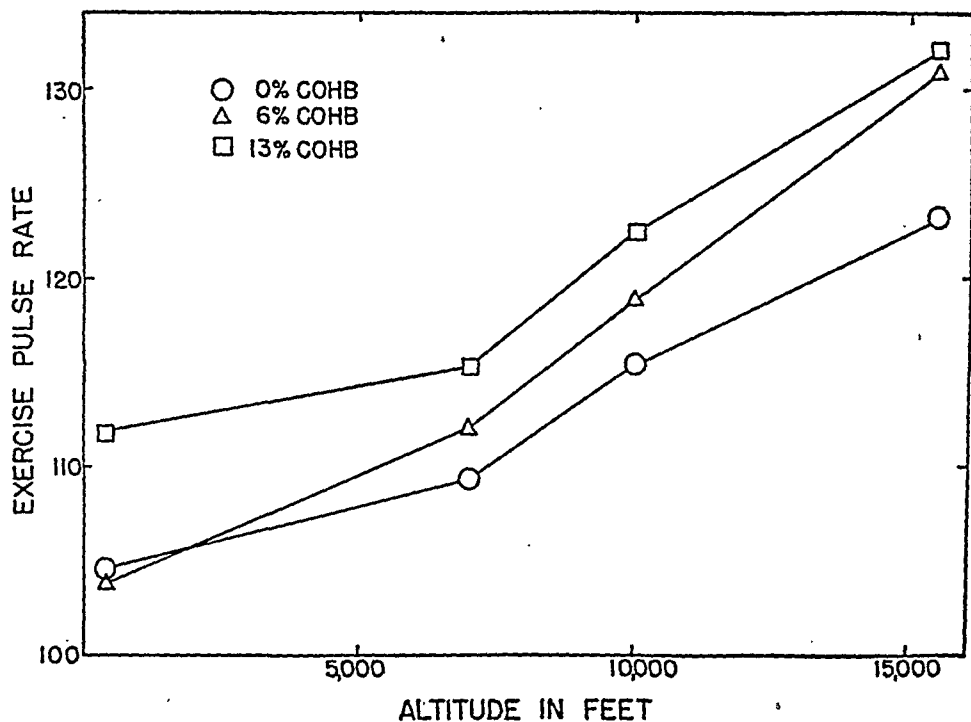


Fig. 3. Effect of altitude and  $\Delta$  COHb on exercise pulse rate. The encircled points are means of 20 values each. The other points are means of 10 values each.

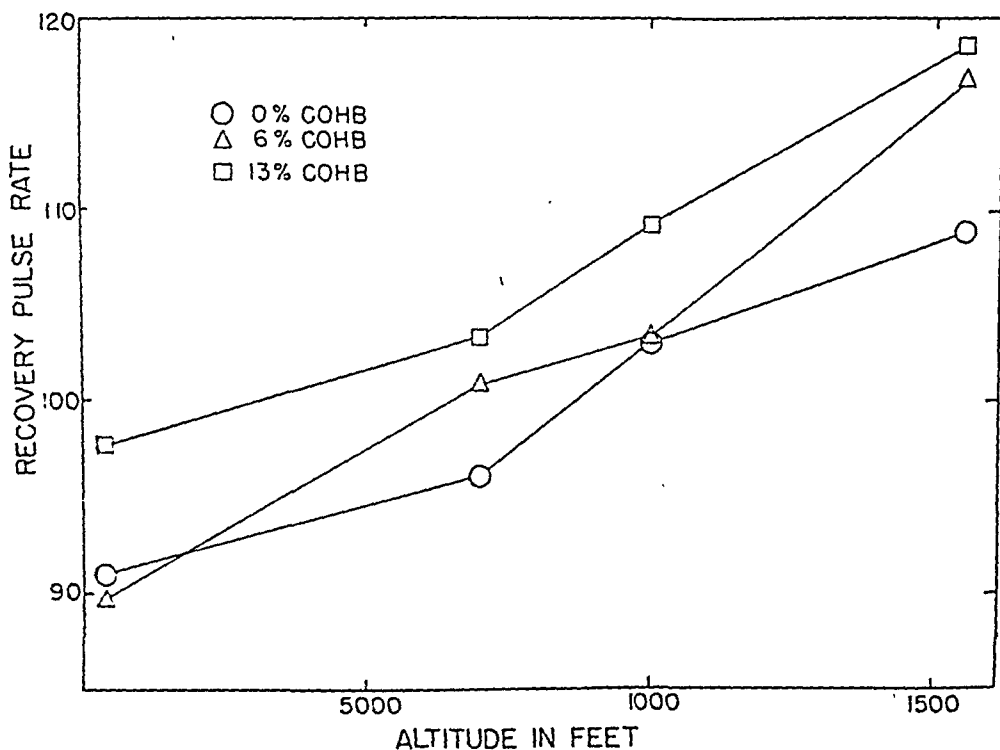


Fig. 4. Effect of altitude and  $\Delta$  COHb on recovery pulse rate. The encircled points are means of 20 values each. The other points are means of 10 values each.

Each point on the "normal" curves (0 per cent  $\Delta$  COHb) is a mean of 20 values and every other point is a mean of 10. The three levels of  $\Delta$  COHb yield roughly parallel curves from 7,000 feet upward for both the exercise pulse (fig. 3) and the recovery I pulse (fig. 4). Assuming that the curves for 0 per cent  $\Delta$  COHb describe the pulse response of normal men to altitude, it is obvious that any given point on the 6 per cent or 13 per cent  $\Delta$  COHb curves corresponds to a higher altitude on the normal curve. These relationships will be considered further under Discussion.

The oximeter data in table 1 are of interest mainly because they justify the use of oxygen-nitrogen mixtures to simulate altitudes in this experiment. The oximeter values obtained during the recovery II period were compared with the data compiled by Henson et al. (7) for the relationship between arterial oxygen saturation and altitude. The close agreement in degree of hypoxia between our simulated altitudes and actual altitudes is illustrated in table 2.

TABLE 2  
*Oximeter values for arterial saturation*

ALTITUDE	ARTERIAL OXYGEN SATURATION	
	Present work	Data of Henson, et al. (7)
<i>feet</i>		
Sea level	95.7	96.0
7,000	91.7	93.0
10,000	86.2	88.5
15,500	79.1	78.5

DISCUSSION. *The assessment of physiological altitude.* The concept of "physiological altitude" has been a provocative one to aviation physiologists. Obviously it would be most desirable to have such a quantitative expression of the integrated effects on altitude tolerance of barometric pressure, blood COHb content, blood cell count, individual metabolic variations, etc. Considering the present state of our knowledge, however, it would seem wise to analyze the concept of physiological altitude and avoid giving it more significance than it truly holds. First, as Lilienthal and Fugitt (8) have pointed out, the various physiological functions have different hypoxia response thresholds. For example, those authors found no change in flicker fusion frequency below 9,000 feet simulated altitude whereas McFarland et al. (9), using visual intensity discrimination, and the present authors, using pulse rate response to exercise, observed effects at 4,000 to 5,000 feet. In the second place, Birren et al. (10) have pointed out that neither can one assume that the response curves for the various physiological functions remain parallel to each other as the altitude is increased. For example, the equivalence between COHb anoxia and altitude anoxia has been determined from the present data (see below) and from the data of McFarland et al. on intensity discrimination (9). At 7,000 feet the COHb-altitude equivalent derived from intensity discrimination is 100 to 150 per cent greater than the equivalent



derived from the pulse rate data, whereas at 15,000 feet both values are nearly the same. Thus it becomes obvious that "physiological altitude" should be based upon a truly representative battery of tests in order to be of much use. But since it is desirable to provide a common scale of comparison for the many factors affecting hypoxia tolerance, we shall express their effects in terms of pressure altitude equivalence. The altitude equivalent of a factor may be defined as the change in pressure altitude which will cause a response equal to that observed when the factor is changed by one unit. This treatment has also been applied to the effects of induced polycythemia (11).

*Pulse rate test of hypoxia tolerance.* Under Methods we have presented a pulse rate test of hypoxia tolerance. While other functions more sensitive to hypoxia have been reported (9), the pulse rate test yields values which probably lie close to a mean physiological response to anoxia and is based on a circulatory response with widespread effects. Critical factors involved in administration of the test are: each subject should have only one hypoxia tolerance test a day and always at the same time of the day; the subjects should be thoroughly indoctrinated in the physical exercise used; and an accurate normal curve for the subjects' response to uncomplicated altitude hypoxia should be determined. With regard to the latter, it should be noted that casual observations on pulse rate form no basis for determining the altitude tolerance or carbon monoxide tolerance of an individual. Such an isolated application of figures 3 and 4 is meaningless. For example, if a subject at 10,000 feet is observed to have a pulse rate of 119, one cannot by reference to figure 3, conclude that his "physiological altitude" is 12,500 feet, and has been raised 2,500 feet by CO or some other factor. The normal pulse rate of this subject at 10,000 feet might be 119. Only if an individual's normal rate response is known, and this response is used as a standard for comparison, can such methods of calculation be used. Since the standard for comparison is determined for each set of experimental conditions, considerable flexibility is possible in the type of exercise used. One can vary the nature and degree of physical stress imposed as long as the conditions are constant within each study. For example, Pace et al., studying the effects of polycythemia on hypoxia tolerance (11) found a treadmill speed of 4 mph. with no grade to be more convenient than the 3 mph. and 2 degree grade used here. In the latter reference the following improvements in the administration of the hypoxia tolerance test are applied. (A) The ten minute "hypoxia rest period" during which the subject breathed a low concentration of oxygen in nitrogen was preceded by a three minute "air rest period" during which the subject stood in position on the treadmill and breathed room air. This gives the observer a check on any considerable changes in the subject's normal standing pulse rate. (B) The "hypoxia recovery period" was reduced to five minutes, which is sufficient with this rate of exercise to assure that the subject's pulse rate has returned to the pre-test base line. (C) In comparing exercise pulse rate means an average of the last four values rather than all five was used. This was found to give a more representative value.

*Statistical considerations of the pulse rate data.* The use of the mean exercise

pulse rate and mean recovery I pulse rate as criteria of COHb-altitude equivalence may be justified on a physiological basis on the classic grounds that the response of an organism to an environmental factor is augmented if the organism is placed under stress. There appears to be statistical justification as well for the use of these two measures in the present case.

In order to examine the relative worth of the four pulse rate measures, Fisher's  $t$  and the probability value  $P$  (12) were obtained for the difference of the mean pulse rate between the various levels of COHb. This was done for each pulse rate measure for 7,000 feet. The exercise pulse rate consistently gives the lowest  $P$  values, and the recovery I gives the next lowest. Since the  $P$  values are very low it may be said that the presence of even 6 per cent  $\Delta$  COHb in the blood affects the pulse rate significantly.

A comparison of the  $t$  and  $P$  values for exercise pulse and recovery I pulse for the other altitudes studied was also calculated. The exercise pulse yields  $P$  values generally lower than those from recovery I pulse. As may be expected, the  $t$  values for the difference between 0 and 6 per cent  $\Delta$  COHb and between 6 and 13 per cent  $\Delta$  COHb are very similar, and are approximately half of the values for the difference between 0 and 13 per cent  $\Delta$  COHb. The latter  $t$  values yield  $P$  values which are all below the one per cent probability level, and the difference in pulse rate under these conditions must be regarded as highly significant. Thus a real effect of CO on pulse rate cannot be doubted, and the pulse rate during exercise and in the first five minutes after exercise are apparently the best quantitative indicators of the degree of such an effect.

The test-retest reliability characteristics of the pulse rate measures are difficult to evaluate properly on so small a group as 10 men. However, some indication of the reliability was afforded by computing the rank-order correlation ( $\rho$ ) of test and retest. The retest was made only under the condition of 0 per cent  $\Delta$  COHb. It appears that the test-retest reliability of the pulse rate measurements may not be high. For this reason the use of group means is necessary if pulse rates are employed in the estimation of COHb-altitude equivalence.

The question of whether exercise pulse alone might be used may be answered in the affirmative. The rank-order correlation coefficients between exercise pulse and recovery I pulse under the various experimental conditions were calculated. The values are fairly high (0.65 to 0.94), so it appears that both exercise pulse and recovery I pulse measure somewhat the same function. Moreover, the use of the recovery I pulse in calculating the COHb-altitude equivalent affords a check on the values obtained by using the exercise pulse.

*COHb-altitude equivalent.* The present problem is one of ascertaining the simultaneous effects of anoxia induced by CO and anoxia induced by low oxygen tensions in the ambient air, and of establishing the equivalence between them. The latter may be expressed as the number of feet increment in pressure altitude required to give the same response as that obtained when blood COHb is increased one per cent, and may be termed the "COHb-altitude equivalent".

In table 3 are presented the values for "altitude equivalence" of COHb as calculated for our data. These values were obtained in the following way. As

pointed out above, the curves in figures 3 and 4 are essentially straight lines between 7,000 and 15,500 feet. Consequently, a least squares fit was drawn to each curve between these two altitudes. The pulse rate responses at 7,000 and

TABLE 3

*Derivation of the mean COHb-altitude equivalent from the pulse rate data in figures 3 and 4*

ALTITUDE SIMULATED	PULSE MEASURE USED	CORRESPONDING ALTITUDE ON NORMAL CURVE WITH		GAIN IN ALTITUDE DUE TO	
		6% $\Delta$ COHb	13% $\Delta$ COHb	6% $\Delta$ COHb	13% $\Delta$ COHb
<i>feet</i>		<i>feet</i>	<i>feet</i>	<i>feet</i>	<i>feet</i>
7,000	Exercise	8,400	10,700	1,400	3,700
7,000	Recovery I	8,400	11,500	1,400	4,500
10,000	Exercise	12,600	14,400	2,600	4,400
10,000	Recovery I	12,500	15,100	2,600	5,100

CONDITIONS FOR WHICH MEAN COHb ALTITUDE EQUIVALENT IS CALCULATED			MEAN COHb-ALTITUDE EQUIVALENT
Simulated altitude	Pulse measure used	Per cent $\Delta$ COHb	
<i>feet</i>			<i>feet/1% <math>\Delta</math> COHb</i>
7,000	Exercise	6 and 13	258
7,000	Recovery I	6 and 13	290
7,000	Exercise and recovery I	6	233
7,000	Exercise and recovery I	13	315
			274
10,000	Exercise	6 and 13	386
10,000	Recovery I	6 and 13	404
10,000	Exercise and recovery I	6	425
10,000	Exercise and recovery I	13	365
			395
7,000 & 10,000	Exercise	6	333
7,000 & 10,000	Exercise	13	312
			323
7,000 & 10,000	Recovery I	6	325
7,000 & 10,000	Recovery I	13	370
			348
Over-all mean			335

10,000 feet were used in calculating the altitude equivalents because the most direct application of the data is in this range. It is presumed that pilots are wearing oxygen masks above 10,000 feet. Furthermore, to calculate the COHb-altitude equivalent at 15,500 feet would have necessitated extending the normal

curve to about 20,000 feet. Obviously it would be difficult and dangerous to obtain data on subjects exercising at this altitude, and extrapolation of the normal curve to this extent does not seem justifiable.

At 7,000 feet the exercise pulse rate on the curve for 13 per cent  $\Delta$  COHb is 116 beats per minute.\* A line drawn through this point parallel to the abscissa intersects the curve for 0 per cent  $\Delta$  COHb at 10,700 feet. Thus, the subjects with 13 per cent  $\Delta$  COHb had a mean pulse rate which was reached by the subjects under normal conditions, i.e., with 0 per cent  $\Delta$  COHb, at an altitude of 10,700 feet. Hence the effect on pulse rate of each 1 per cent  $\Delta$  COHb was equivalent to increasing the pressure altitude by 269 feet. All values in table 3 were obtained by this method.

It may be noted in table 3 that the mean altitude increment per 1 per cent  $\Delta$  COHb is 274 feet at 7,000 feet altitude, and 395 feet at 10,000 feet altitude.

TABLE 4  
*COHb-altitude equivalence for various physiological functions*

SOURCE	CRITERION	ALTITUDE RANGE STUDIED	COHb-ALTITUDE EQUIVALENT
		<i>feet</i>	<i>ft. per 1% <math>\Delta</math> COHb</i>
Lilienthal and Fugitt (8).....	Flicker fusion frequency	5,000 to 6,000	400 to 500
McFarland, Roughton, Halperin, and Niven (9).....	Visual intensity discrimination	7,000 10,000	500 to 700 400 to 600
Pitts and Pace (above).....	Exercise pulse	7,000 to 10,000	300 to 400
Vollmer, King, Fisher and Birren (13).....	Flicker fusion freq., body sway and red visual fields	10,000 15,500	0 0

This suggests that the effect of CO on altitude tolerance increases as altitude is increased, but this apparent divergence probably is not significant. Thus it is not inconsistent with the overall accuracy of the method to use a mean COHb-altitude equivalent for the altitude range under consideration.

No consistent difference is apparent between the mean values for exercise and recovery or between the mean values for 6 per cent  $\Delta$  COHb and 13 per cent  $\Delta$  COHb. Therefore, it appears that the overall mean of 335 feet increase in pressure altitude for each 1 per cent increase in blood COHb is a value for the COHb-altitude equivalent which may be applied at any altitude up to 10,000 feet, and with any COHb increment up to 13 per cent.

In table 4 is presented the COHb-altitude equivalent as determined by a variety of criteria. Where possible, the values given are those for the same range of altitude and  $\Delta$  COHb studied in the present experiments. Aside from the data

of Vollmer et al. (13) the general agreement of the values is gratifying when one considers the complexity of the physiological picture of anoxia.

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#### SUMMARY

1. The effects of various levels of blood COHb and of various simulated altitudes on the physiological response to exercise were observed in a group of ten normal men.

2. The COHb conditions were: 0, 6, and 13 per cent  $\Delta$  COHb at simulated altitudes of 0, 7,000, 10,000 and 15,500 feet. These altitudes were simulated by having the subjects breathe appropriate mixtures of oxygen and nitrogen so as to produce arterial oxygen saturations corresponding to those found at the altitudes listed above.

3. The physiological response was examined by measurement of pulse rate, respiratory rate, and minute volume of respiration. These functions were measured each minute during a ten minute rest period, a five minute period of mild exercise, and a ten minute exercise recovery period. Of these measures, the mean exercise pulse rate and the mean pulse rate during the first five minutes following exercise exhibited the closest correlation with the experimental variables, i.e., blood COHb and ambient oxygen tension.

4. On the basis of the changes in pulse rate it was possible to derive a quantitative estimate of the effect of blood COHb on hypoxia tolerance. The increment in response per 1 per cent increase in blood COHb was equal to that obtained by raising a normal group 335 feet in pressure altitude. This value was determined for the altitude range of 7,000 to 10,000 feet and for increases in blood COHb of at least 13 per cent.

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# THE INCREASE IN HYPOXIA TOLERANCE OF NORMAL MEN ACCOMPANYING THE POLYCYTHEMIA INDUCED BY TRANSFUSION OF ERYTHROCYTES<sup>1</sup>

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The marked dependence of the hemoglobin content of the blood, whether measured in terms of oxygen carrying capacity or erythrocyte count, on the partial pressure of oxygen in the ambient air has been studied since the time of the original hypothesis of Bert (1). The recent paper of Hurtado, Merino and Delgado (2) provides an excellent summary of the effects of low oxygen pressure on hemopoiesis. Not only has the increase in blood hemoglobin content with decrease in ambient oxygen pressure been observed, but also a decrease in hemoglobin with an increase in ambient oxygen has been noted (3). The rôle of this phenomenon in the process of acclimatization to high altitudes has been the subject of considerable research and speculation, frequently leading to divergent conclusions. Dill (4) believes that a close balance exists between the advantages and disadvantages of an increased amount of hemoglobin at high altitudes, whereas Van Liere (5) lists the increased hemoglobin as one of the important compensatory mechanisms comprising the process of high altitude acclimatization. More specifically, Dorrance et al. (6) demonstrated the improvement of work performance under conditions of anoxia in rats treated with cobalt so as to produce polycythemia. On the other hand, Wetzig and D'Amour (7), using the response to a bell of rats under conditions of anoxia, could observe no significant effect of polycythemia induced by injection of red cell suspensions in saline. Campbell (8) concluded first that the increased hemoglobin content had no effect on tissue oxygen tension and hence was not essential to acclimatization, and later (9) that the tissue oxygen tension was improved with an increased hemoglobin content.

The feasibility of producing an uncomplicated polycythemia in normal men by transfusion of matched erythrocytes suspended in saline was demonstrated in a preliminary report from this laboratory (10). Pitts and Pace (11) have described a test procedure utilizing the pulse rate during moderate exercise for the comparison of the relative tolerance of groups of men to hypoxia. This procedure has been used in the present study to examine the effect of transfusion polycythemia on the hypoxia tolerance of young men.

**EXPERIMENTAL METHODS.** Ten healthy young men, all 18 years old, were selected from volunteer naval personnel following a thorough physical examination. The ten subjects selected had type A, Rh positive blood. They were

<sup>1</sup> The opinions or conclusions contained in this paper are those of the authors. They do not necessarily reflect the views or endorsement of the Navy Department.

divided into two groups of five men each as nearly equated as possible in body structure and physiological response to exercise. The men lived in the laboratory and were allowed the freedom of the vicinity during the day, except at the time of scheduled tests. Their meals consisted of the standard Navy ration. All subjects were in bed by 2200. The men were given liberty on Saturday night and returned to the laboratory by 1800 on Sunday. It was found possible by these means to control adequately the general physical condition of the subjects over a ten week period without undue hardship. The importance of such general control has been found to be great in the attainment of reproducible results with human subjects.

For the first few days following their arrival the men were instructed in the routine of the experiment and briefly in its purpose. They were also indoctrinated in walking on the power-driven treadmill. A standard speed of 4.0 miles per hour with no grade was used for all treadmill experiments. As described by Pitts and Pace (11) the test for hypoxia tolerance was a standardized and carefully administered physical stress. The test consisted of four distinct periods. (A) An air rest period that lasted three minutes, during which the subject breathed room air while standing in place on a motionless treadmill; (B) a hypoxia rest period that lasted ten minutes, during which the subject breathed the desired oxygen-nitrogen mixture while standing; (C) a hypoxia exercise period that lasted five minutes during which the subject walked on the treadmill while breathing the oxygen-nitrogen mixture; (D) a hypoxia recovery period that lasted five minutes, during which the subject continued to breathe the oxygen-nitrogen mixture while standing in place on the treadmill.

The entire test lasted 23 minutes. Pulse rate was determined by palpation of the brachial artery for 30 second periods each minute of the test. Readings of the arterial oxygen saturation were obtained once a minute by means of a Millikan oximeter. The oxygen-nitrogen mixture was inspired through a standard Navy demand regulator and rubber mouthpiece, and the expired air was collected in a Tissot type gasometer. The respiratory rate was obtained each minute by making a 30 second count of the motions of the gasometer scale. Minute volume of respiration was recorded each minute from the gasometer scale.

The results for each function were expressed as the mean values for each period of the hypoxia tolerance test. For example, four pulse rate values were obtained: the air rest pulse rate, as the mean of the first three minutes of the test during which time the subject breathed room air; the hypoxia rest pulse rate, as the mean of the last five minutes of the period of breathing the oxygen-nitrogen mixture while standing; the hypoxia exercise pulse rate, as the mean of the last four minutes of the exercise period; and the hypoxia recovery pulse rate, as the mean of the last four minutes of the recovery period. Figure 1 illustrates a typical test run, together with the mean values obtained.

Because of the length of the test and also to allow complete recovery from hypoxia, only one test was run on each individual per day. To avoid the effect of diurnal rhythms, each subject was tested at approximately the same time of day. Three mixtures of oxygen and nitrogen were used in the experiment:



16 per cent oxygen and 84 per cent nitrogen, 14 per cent oxygen and 86 per cent nitrogen, and 11 per cent oxygen and 89 per cent nitrogen. The partial pressure of oxygen in these mixtures corresponded to that at altitudes of 7,000 feet, 10,000 feet and 15,500 feet respectively (12). In addition, on Saturday of each week the test procedure was run with each subject breathing room air (21 per cent oxygen) for the entire test. On each Monday morning alveolar air samples were obtained from the subjects and analyzed for oxygen and carbon dioxide. Samples of arterialized venous blood were taken from the superficial veins of the back of the hand by the technic of Goldschmidt and Light (13) and were analyzed for oxygen

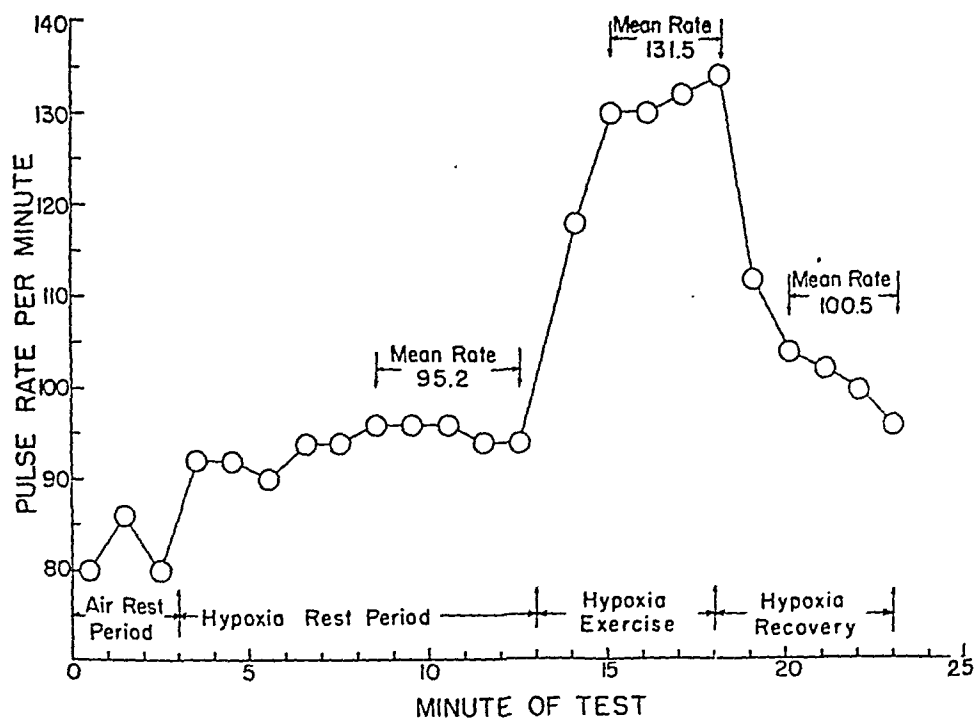


Fig. 1. Hypoxia tolerance test on one subject. The subject breathed 11 per cent oxygen in nitrogen. The various periods of the test are shown, as are the mean pulse rates calculated for each period.

capacity, oxygen content and plasma  $\text{CO}_2$  content by means of the Van Slyke and Neill manometric apparatus. The plasma pH was measured directly by means of a glass electrode pH meter. Hematocrit values and reticulocyte counts were also obtained on these blood samples. These alveolar air and blood studies were made both at ground level and after exposure to a simulated altitude of 15,500 feet for one hour. Overnight urine specimens were collected routinely for microscopic urine examination, urine pH, and total urine pigment by the method of Heilmeyer (14).

The test routine was continued for two weeks to obtain a base level. On days 14, 15, 16 and 17, five of the subjects received transfusions of 500 cc. of a 50 per cent suspension in dextrose and saline solution of unwashed, group A erythrocytes which had been obtained no longer than 24 hours before injection. Individual

cross-matchings between the subject's serum and the red cells to be injected were performed immediately before each transfusion. Each erythrocyte transfused subject received a total of 1000 cc. of red cells. Aside from one man who suffered a mild transitory urticaria following his first transfusion, the transfusions were very well tolerated and there were no subjective complaints. The other five subjects ("control group") received transfusions of 500 cc. of dextrose and

TABLE 1

*Mean values for biochemical data obtained on the control group and transfused group while breathing room air and during exposure in a low pressure chamber to a simulated altitude of 15,500 feet*

DAY OF EXP'T	HEMATOCRIT VALUES (PER CENT)		BLOOD O <sub>2</sub> CAPACITY (VOL. PER CENT)		ARTERIAL O <sub>2</sub> CONTENT (VOL. PER CENT)		ARTERIAL PLASMA CO <sub>2</sub> CONTENT (VOL. PER CENT)		ARTERIAL PLASMA pH		ALVEOLAR O <sub>2</sub> PRESSURE (MM. MERCURY)		ALVEOLAR CO <sub>2</sub> PRESSURE (MM. MERCURY)	
	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.
A. Breathing room air														
1	46.8	46.6	21.1	20.1	19.5	19.2	55.9	54.8	7.46	7.46	103.5	106.5	40.5	38.8
13	45.9	45.8	20.2	19.8	19.3	19.1	54.4	55.8	7.46	7.45	101.1	105.9	39.8	38.6
14-17	Transfusions given during this period													
18	47.6	58.3	20.9	24.4	19.7	23.5	58.4	54.9	7.46	7.45	103.0	101.1	40.9	41.0
25	46.0	55.3	20.5	24.3	19.4	22.8	57.4	56.0	7.46	7.45	99.7	94.8	39.9	42.3
32	45.5	52.6	20.6	23.1	19.7	22.2	56.9	57.2	7.45	7.45	97.6	89.4	41.4	44.2
39	45.7	51.2	20.7	22.7	19.7	21.8	57.2	57.1	7.47	7.47	95.8	92.7	41.5	43.6
46	46.5	48.8	20.9	21.6	20.0	20.4	57.8	57.0	7.45	7.45	96.2	90.9	41.6	43.2
53	45.5	47.2	20.7	21.2	19.6	19.9	57.8	58.5	7.47	7.47	99.1	92.6	41.5	43.4
60	45.4	45.7	20.7	20.3	19.8	19.4	56.9	57.8	7.47	7.48	98.0	86.9	41.2	44.5
64	46.0	46.7	20.9	20.8	20.3	19.8	58.4	57.1	7.47	7.48	98.3	89.1	39.6	42.3
74	47.1	46.4	21.1	20.3	—	—	—	—	—	—	—	—	—	—
B. During exposure in a low pressure chamber to a simulated altitude of 15,500 feet														
4-5	46.0	46.1	20.4	19.9	13.6	13.2	55.9	54.8	7.47	7.47	37.6	39.7	32.9	32.9
7-8	46.3	45.3	20.6	19.7	15.0	13.8	55.4	53.4	7.51	7.50	42.2	41.4	33.5	33.3
14-17	Transfusions given during this period													
21-22	45.6	56.5	20.4	24.6	14.2	17.5	56.7	56.2	7.49	7.48	39.8	39.3	35.0	34.2
28-29	46.7	54.3	21.0	24.3	14.4	17.3	56.6	56.1	7.52	7.53	39.8	37.6	35.2	35.6
35-36	45.3	52.0	20.4	23.2	15.2	16.9	55.1	55.8	7.53	7.53	39.3	38.1	34.4	35.0
42-43	46.0	50.0	20.4	22.2	13.9	15.1	57.1	56.5	7.53	7.53	39.0	36.9	35.0	36.1
49-50	45.1	48.3	20.5	21.5	14.1	14.8	57.2	56.0	7.51	7.51	37.9	36.7	35.4	36.2
56-57	44.3	46.2	20.0	20.7	14.2	15.3	55.7	54.1	7.50	7.50	40.8	37.9	34.0	35.4

saline solution simultaneously with the test group. Arrangements were made so that none of the subjects knew whether he had received erythrocytes or dextrose and saline alone.

The test routine was then resumed and continued for the ensuing two months.

RESULTS. In table 1-A are listed the mean values for the control group obtained from blood and alveolar air studies made on samples taken while the subjects were breathing room air. The data from comparable samples taken while

the subjects were at a simulated altitude of 15,500 feet in a low pressure chamber are presented in table 1-B. It may be seen that the hematocrit values and the blood oxygen capacity were markedly increased by the transfusion. Calculation of the ratio of hematocrit to oxygen capacity yields the same value for the transfused group before and after transfusion, indicating that all of the cells injected were functional. The mean ratios for both control and transfused groups were in agreement with that found by Dill, Edwards and Consolazio (15).

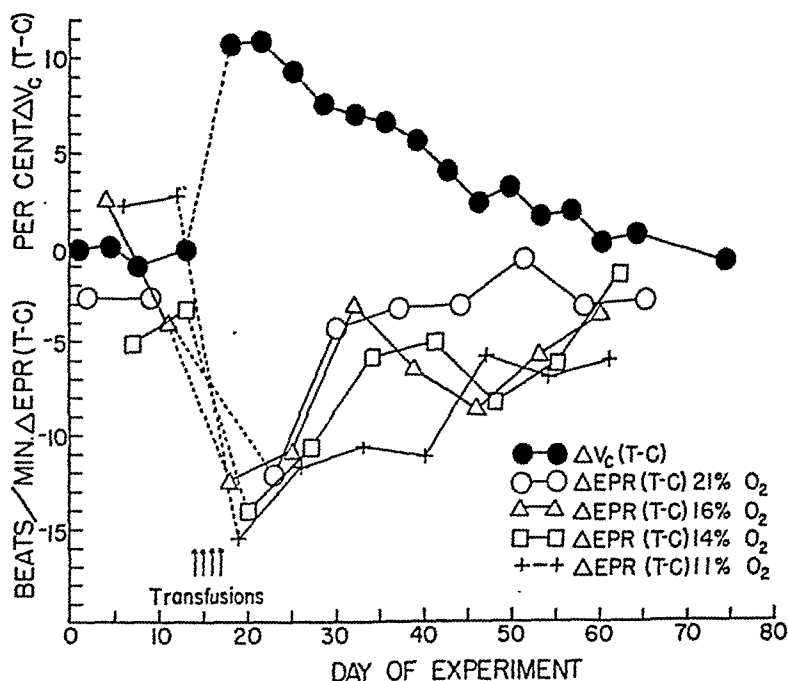


Fig. 2. The uppermost curve (solid circles) represents the difference ( $\Delta V_c$ ) of hematocrit values between the transfused group (T) and the control group (C). The four lower curves represent the difference in exercise pulse rate ( $\Delta E.P.R.$ ) between the transfused group and the control group while breathing the various mixtures of oxygen and nitrogen. The four transfusions of 500 cc. each are indicated by arrows.

The polycythemia decreased linearly, and the hematocrit values returned to normal approximately 50 days after the transfusions. This is brought out by figure 2, where the difference in mean hematocrit value between the transfused group and the control group is plotted as a function of days.

The oxygen content measured on arterialized venous blood is given in table 1. The content of the transfused group rose proportionately with the oxygen capacity both at ground level and at altitude so that the percent saturation of hemoglobin with oxygen remained essentially constant. The alveolar oxygen tension of the transfused group was lower than that of the control group following the transfusion; however, it remained lower even after the disappearance of the polycythemia so that the significance of the lowering is debatable.

The possibility of a disturbance in acid base balance induced by the polycythemia was examined by measuring the plasma  $CO_2$  content and pH and the

TABLE 2

Mean values for physiological data obtained from the hypoxia tolerance test on the control group and transfused group while breathing various mixtures of oxygen and nitrogen

DAY OF EXP'T	STANDING PULSE RATE (BEATS/MIN.)		EXERCISE PULSE RATE (BEATS/MIN.)		RECOVERY PULSE RATE (BEATS/MIN.)		EXERCISE RESPIRATORY MIN. VOL. (LITERS/MIN.)		EXERCISE RESPIRATORY RATE (BREATHS/MIN.)		EXERCISE ARTERIAL O <sub>2</sub> SATURATION (PER CENT)	
	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.	Contr.	Trans.
A. 21 per cent oxygen in nitrogen (room air)												
2	92.9	85.6	113.2	110.5	100.6	89.0	24.6	25.7	23.5	25.6	97.8	96.4
9	90.3	89.1	113.6	110.9	99.5	92.8	24.9	25.5	23.0	24.7	99.0	100.1
14-17	Transfusions given during this period											
23	89.7	81.9	112.3	100.2	93.7	86.4	27.5	24.5	25.1	27.2	98.7	98.4
30	98.6	92.7	117.0	112.7	101.3	101.6	25.3	24.5	26.8	26.7	93.9	93.8
37	87.8	89.8	111.3	108.1	95.0	92.4	25.5	22.8	26.2	24.1	96.5	96.9
44	95.8	94.2	116.0	112.9	101.1	99.4	24.9	24.4	25.7	26.0	97.0	96.8
51	97.1	96.7	115.0	114.4	99.9	99.6	26.3	25.2	25.9	26.4	96.6	95.6
58	97.2	93.8	120.9	117.8	101.9	99.3	27.3	25.1	26.9	26.9	98.6	97.2
65	99.6	95.4	121.3	118.5	107.5	100.6	28.5	26.0	27.2	27.5	92.6	93.8
B. 16 per cent oxygen in nitrogen (7,000 ft. equivalent altitude)												
4	90.3	87.8	115.3	117.8	93.5	89.4	24.8	25.4	21.2	25.2	87.9	89.1
11	95.6	93.9	117.4	113.3	100.2	94.1	26.1	25.9	23.2	24.7	91.9	90.0
14-17	Transfusions given during this period											
18	94.2	87.5	119.6	107.2	101.0	90.2	28.4	26.6	24.8	25.1	92.0	88.0
25	97.8	89.2	124.3	113.5	104.3	94.3	27.6	24.4	26.0	27.0	92.7	89.5
32	97.4	91.2	121.1	118.0	105.1	95.6	26.6	23.8	25.6	24.8	89.1	89.8
39	92.7	89.6	119.8	113.3	97.6	96.2	27.6	24.6	26.3	24.9	90.7	90.0
46	97.2	90.4	121.8	113.2	101.5	93.3	27.3	26.0	27.3	24.4	91.9	90.8
53	92.6	89.1	121.6	115.9	96.8	91.0	27.9	26.2	27.2	26.7	90.8	91.8
60	91.5	86.8	120.5	116.9	98.2	90.0	28.1	25.8	27.1	26.2	92.5	92.0
C. 14 per cent oxygen in nitrogen (10,000 ft. equivalent altitude)												
7	100.2	93.4	126.8	121.7	107.1	97.3	28.1	27.7	24.5	24.0	85.3	84.4
13	90.9	89.6	120.1	116.8	96.9	91.5	27.4	26.9	24.5	24.2	88.4	88.0
14-17	Transfusions given during this period											
20	98.6	85.9	127.0	113.0	106.3	89.7	28.4	27.9	25.2	25.2	86.5	80.5
27	98.8	90.3	125.4	114.7	101.6	93.4	28.7	27.1	25.6	26.7	85.5	83.0
34	99.6	93.8	126.5	120.7	107.0	95.6	28.9	26.4	26.3	26.6	86.1	87.8
41	98.4	93.8	127.6	122.6	103.8	97.5	29.8	27.3	27.6	24.1	77.7	82.2
48	99.4	91.5	127.1	118.9	103.9	96.7	27.5	28.2	27.5	25.9	85.0	85.5
55	94.4	86.6	126.1	120.0	98.6	88.6	30.8	27.3	27.4	25.2	87.5	85.7
62	97.1	88.7	126.5	125.1	101.2	95.5	29.6	27.7	26.3	26.9	85.6	87.3
D. 11 per cent oxygen in nitrogen (15,500 ft. equivalent altitude)												
6	103.2	95.8	136.3	138.5	110.4	100.8	31.8	33.0	24.5	27.4	71.6	71.4
12	97.0	94.0	133.0	135.7	104.9	97.4	31.5	33.5	25.8	27.4	76.0	74.1
14-17	Transfusions given during this period											
19	104.0	87.8	143.3	127.8	112.7	94.0	35.2	32.4	25.2	25.7	70.4	68.5
26	109.0	94.4	144.5	132.8	114.7	101.7	34.6	31.6	27.8	27.9	74.9	70.8
33	105.7	94.8	140.5	129.9	112.0	103.2	34.3	31.1	27.9	27.5	75.2	75.3
40	106.8	93.9	140.0	128.9	114.2	96.7	35.2	31.0	27.9	27.5	76.2	69.1
47	104.6	102.7	142.5	136.7	113.1	102.2	35.1	32.8	27.6	30.1	70.7	78.6
54	103.4	94.9	144.1	137.2	116.2	100.6	34.3	33.6	28.7	26.1	76.2	77.3
61	103.3	93.4	143.2	137.2	111.7	99.3	37.6	33.4	30.5	28.6	72.5	77.5

alveolar CO<sub>2</sub> tension. As may be seen in table 1-A there was no difference between the control group and transfused group in this regard. Both groups exhibited the characteristic alkalosis associated with hypoxia as indicated by the plasma pH and alveolar CO<sub>2</sub> data in table 1-B.

In table 2 are shown, for the control group and the transfused group, the mean values of the standing pulse rate, the exercise pulse rate and the recovery pulse rate while breathing the four mixtures of oxygen and nitrogen used in this study. It is evident that the mean pulse rate of the transfused group was lowered following the transfusion, and gradually rose as time went on. In order to smooth out some of the variability due to external factors, the pulse rate changes were expressed as the difference between the transfused group and the control group, and the difference was plotted against time. The exercise pulse rate showed the most consistent and largest changes, as noted previously (11), and the difference

TABLE 3

*Total reticulocyte volume in the blood of the five control group subjects and five transfused group subjects following the transfusion period*

DAY OF EXP'T	CONTROL GROUP SUBJECTS TOTAL RETICULOCYTES (CC.)						TRANSFUSED GROUP SUBJECTS TOTAL RETICULOCYTES (CC.)						MEAN DIFFERENCE, CONTROL - TRANSFUSED
	P. O. L.	L. O. E.	C. H. A.	S. T. O.	S. L. A.	Mean	W. A. T.	H. E. N.	B. E. N.	B. E. A.	S. T. E.	Mean	
18	52.1	9.2	39.2	39.6	3.0	28.6	3.1	29.6	12.6	3.0	41.0	17.9	10.7
25	30.6	38.0	11.7	23.0	23.3	25.3	2.9	2.8	5.9	0.0	11.1	4.5	20.8
32	20.6	27.3	2.3	14.3	8.4	14.6	0.0	2.7	48.7	0.0	2.6	10.8	3.8
39	21.0	2.2	13.3	3.0	0.0	7.9	2.8	0.0	5.3	2.7	2.6	2.7	5.2
47	6.9	2.2	0.0	9.0	0.0	3.6	0.0	2.5	2.5	2.6	0.0	1.5	2.1
55	23.1	4.4	6.8	0.0	0.0	6.9	2.6	23.9	4.7	5.2	2.4	7.8	-0.9
62	2.2	4.4	0.0	0.0	0.0	1.3	0.0	6.9	0.0	12.5	2.3	4.3	-3.0

between transfused and control groups is plotted in figure 2 on the same time scale as the hematocrit differences.

Mean values for exercise respiratory minute volume and rate are listed in table 2; however, no significant differences could be detected between the transfused group and control group in any of the respiratory measures. The arterial oxygen saturation during exercise as measured by the Millikan oximeter is also given in table 2. There was a tendency for the saturation of the transfused group to be lower than that for the control group for a short period following the transfusion, but the difference was not significant. The measure was of value in assuring that the subjects were exposed to the desired degree of hypoxia, and the data were obtained primarily with this object in mind.

Reticulocyte counts, as number of reticulocytes per thousand erythrocytes, were obtained at intervals following the transfusions on both the control and transfused groups. These data were expressed as the total cubic centimeters of reticulocytes in the blood of each subject by multiplying the count by the hemato-

crit value and the blood volume. The results are shown in table 3. Both groups exhibited mean values higher than the normal value of 8 to 12 cc. in the period immediately following the transfusion. The transfused group appeared to have a consistently lower reticulocyte volume than the control group until the normal hematocrit was restored. No explanation of the relatively high reticulocyte volume in both groups for the period shortly after the transfusions can be given. Unfortunately, no counts were made on the subjects beforehand, so it cannot be said whether or not the effect was caused by the intermittent exposure to hypoxia almost daily in the course of the experiment.

The measurements of the total urinary pigment output per hour revealed no difference between the transfused and control groups either before or after the transfusions.

**DISCUSSION.** From the results of this experiment it is obvious that a substantial polycythemia may be induced in normal men by the transfusion of erythrocytes without untoward results. The mean hematocrit value for the transfused group was increased from 46.2 to 58.3 per cent. Calculation of the expected hematocrit level following injection of 1000 cc. of erythrocytes into the vascular system yielded a value of 54.9 per cent. It appears, therefore, that a slight transitory decrease in plasma volume must have occurred as a result of the transfusions, possibly in an effort to compensate for the increased blood volume. That this decrease in plasma volume occurred is substantiated by the simultaneous observation of a slight increase in plasma proteins and the plasma specific gravity in the transfused subjects during this period. It is of interest to note that the control group exhibited evidence of hemoconcentration on day 18, following the injection of 2000 cc. of glucose and saline solution during the preceding four days.

The presence of the extra red cells apparently did not embarrass the lung gas-exchange mechanism because the arterial blood was saturated to the same degree, or possibly slightly less, in the transfused group as in the control group under conditions of both normal and low ambient oxygen pressure. Furthermore, no significant difference could be detected in the respiratory pattern of the transfused group before and after transfusion. The oxygen content of the arterial blood of the transfused group was increased by approximately 23 per cent as a result of a similar percentage increase of red cells. In other words, there was no indication of a shift in the position of the oxygen dissociation curve of the blood as a result of the transfusion. This agrees essentially with the finding of Keys, Hall and Guzman Barron (16) and of Aste-Salazar and Hurtado (17) that there is no shift to the left of the oxygen dissociation curve in the case of persons acclimatized to altitude, but possibly some shift to the right.

The use of the pulse rate during exercise under conditions of hypoxia, as a criterion of the physiological advantage conferred upon the transfused group by the increased oxygen content of the blood, appears to be reasonable and valid in so far as the cardiovascular system is concerned. As pointed out by Pitts and Pace (11), the exercise pulse rate may be considered to vary as a straight line function of altitude between 7,000 and 15,500 feet. Hence, it was possible to

estimate the decrease in hypoxia tolerance associated with various levels of carboxyhemoglobin in the blood in this altitude range by observing the change in exercise pulse rate. In the present experiments a similar approximation can be made, and this was done for the exercise pulse rates for the first three weeks following the transfusion. Thus, with regard to the increase in exercise pulse rate with increase in altitude, the displacement of the curve for the transfused group from the curve for the control group represents the increase in hypoxia tolerance of the former in terms of feet of altitude. For example, in the first week following the transfusion the exercise pulse of the transfused group at a simulated altitude of 15,500 feet corresponded to that of the control group at 10,300 feet. Therefore, as judged by the pulse rate criterion the "physiological altitude" of the transfused group was decreased 5,200 feet as a result of the transfusion. As

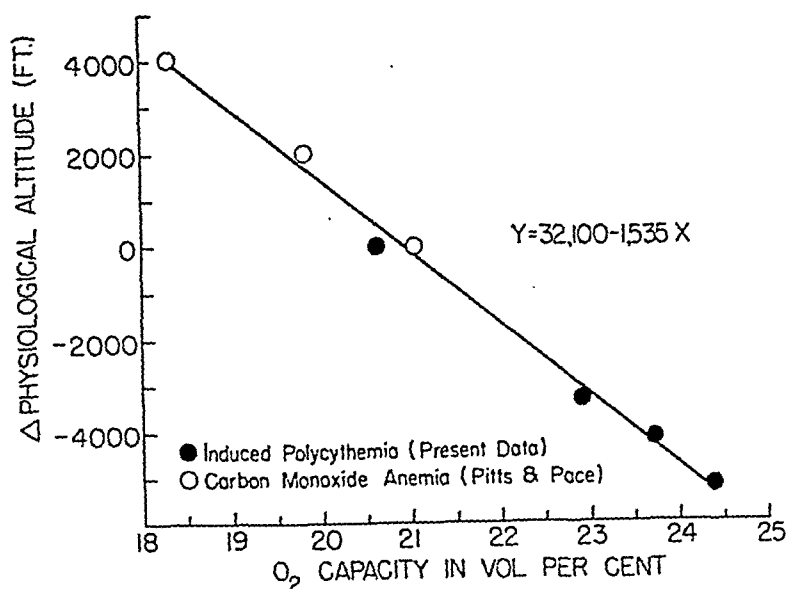


Fig. 3. The increase or decrease ( $\Delta$ ) in physiological altitude, as determined by changes in the exercise pulse rate under conditions of hypoxia, when the oxygen capacity of the blood is decreased by CO poisoning or increased by transfusion of erythrocytes.

the oxygen capacity of the blood of the transfused group decreased to normal, the exercise pulse rate increased toward that of the control group. The correlation coefficient between oxygen capacity and change in exercise pulse rate (control group mean minus transfused group mean) for all conditions tested was 0.77 while for the particular case of subjects breathing 11 per cent oxygen in nitrogen the coefficient reached 0.89.

In order to make a more direct comparison, the improvement in hypoxia tolerance expressed as a drop ( $-\Delta$ ) in physiological altitude of the transfused group for the three weeks following the transfusion was plotted against the blood oxygen capacity. The data of Pitts and Pace (11) on the opposite side of normal were also included, and the resultant relationship is shown in figure 3. The agreement is good and serves as further substantiation of the exercise pulse rate method for determining tolerance to hypoxia, as well as a demonstration of the

beneficial effect of an increased number of red cells in hypoxia tolerance. It is to be emphasized that the exercise pulse rate is useful only as a group mean with adequate controls for comparison. Individual values of exercise pulse rate cannot be used as criteria of hypoxia tolerance because of wide fluctuations even under carefully regulated conditions (11).

As pointed out first by Asmussen and Chiodi (18), the stimulus for respiratory movements appears to be the oxygen tension in the arterial blood rather than the oxygen content. Thus in partial carbon monoxide poisoning where large decreases in oxygen content can occur with very small changes in oxygen tension the respiration is relatively unaffected. The same is found to be the case with the artificial polycythemia in the present study. A large increase in oxygen content of the blood occurred with little change in arterial blood oxygen tension, and the respiration was unaffected.

The opposite seems true for the heart rate, as shown by the experiments both with carbon monoxide poisoning and with artificial polycythemia. The heart rate appears to be profoundly affected by the blood oxygen content. Even at sea level, where the blood is nearly saturated, the exercise pulse rate of the transfused groups showed a significant reduction following transfusion. Asmussen and Chiodi (18) observed that the heart rate is increased by a lowered oxygen content of the blood; however, their measurements indicated that the cardiac output does not increase, so that the stroke volume must be decreased. On the other hand they observed an increased cardiac output when the blood oxygen tension was lowered. Placing the greatest weight upon cardiac output these authors concluded that the circulation, as well as the respiration, is stimulated by changes in oxygen tension rather than in oxygen content. It may be argued that the tissue oxygen tension is regulated to some degree by the oxygen content of the blood because oxygen consumption is assumed to be constant for a given tissue, and the greater the oxygen content of the blood the less the tension will fall for the removal of a constant amount of oxygen by the tissues. Following this line of thought it would appear that the exercise pulse rate reflects ultimately the tissue oxygen tension and as such is a valuable measure. In any case the exercise pulse rate shows good correlation with the oxygen content of the blood (11). It is, therefore, a measure of tolerance for hypoxia.

The hypoxia tolerance of the transfused group was shown to have been greater than that of the control group by an amount equivalent to 5,200 feet of altitude. Furthermore, changes in altitude from sea level to 5,000 feet have no perceptible physiological effects. Therefore, it may be concluded that the transfused group at an altitude of 10,200 feet would be physiologically equivalent to the control group at sea level. This conclusion is confirmed by the exercise pulse rate data given in table 2. It is of interest that the mean hematocrit value of the transfused group immediately after transfusion was within the range given by Hurtado, Merino and Delgado (2) for residents at an altitude of 10,000 feet.

As mentioned above, the duration of the polycythemia was approximately 50 days. This is about half the time of the best estimates of the life of transfused red cells (19) as determined by the agglutination technic (20). Thus either the



transfused cells were destroyed more rapidly than normally in this experiment, or the production of new cells was temporarily decreased. Although fecal urobilinogen excretion studies were not made, in view of the lack of change in total urine pigment output it does not appear that red cell destruction was increased appreciably. On the other hand, the transfused group exhibited a tendency toward a lower reticulocyte volume than did the control group following the transfusion, as shown by table 3. Therefore, it may be tentatively concluded that normal erythropoiesis was inhibited to some degree by the presence of the injected erythrocytes.

#### SUMMARY AND CONCLUSIONS

1. An artificial polycythemia was induced in a group of five normal young men by the transfusion of 2000 cc. of a 50 per cent suspension of compatible erythrocytes in glucose and saline solution at a rate of 500 cc. per day for four days. The mean hematocrit value for the group was increased from 46.2 to 58.3 per cent. The polycythemia was well tolerated and lasted for approximately 50 days.

2. Biochemical and physiological studies were made on this group and compared with similar studies made simultaneously on a control group of five men who had received similar transfusions of 2000 cc. of glucose and saline solution alone.

3. The arterial oxygen content of the erythrocyte transfused subjects was increased proportionately to the increase in oxygen capacity, thus the per cent saturation of their blood with oxygen was the same as that of the control subjects. This was true both at sea level and at a simulated altitude of 15,500 feet.

4. Tolerance to hypoxia was estimated on the basis of the pulse rate during exercise under conditions of lowered oxygen tension. It was found that the exercise pulse rate of the transfused group dropped sharply in the week following the transfusions and then gradually returned to the level of the control group as the cell count decreased. It was possible to estimate from these measurements that during the first week of polycythemia the transfused subjects when at a simulated altitude of 15,500 feet gave the pulse rate response of a normal group at only 10,300 feet.

5. It is concluded that the polycythemia induced artificially in this experiment and the polycythemia which occurs during acclimatization to high altitudes are very similar. Therefore, the latter must play an important part in the attainment of acclimatization, and may represent the bulk of the acclimatization process.

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# THE RELATION OF BLOOD VOLUME REDUCTION TO MORTALITY RATE IN HEMORRHAGIC AND TRAUMATIC SHOCK IN DOGS<sup>1,2</sup>

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Reduction of the circulating blood volume is undoubtedly one of the most important changes occurring in traumatic shock. Indeed, many investigators believe that shock and hemorrhage are identical (see 1). The evidence put forth by Blalock (2) and Parsons and Phemister (3) suggests that in severe muscle injury the local loss of blood and fluid is sufficient by itself to cause shock and death. The important question arises as to whether the amount of fluid lost from the blood stream following a moderate degree of muscle trauma is also adequate to account for the ensuing fatal syndrome. In order to ascertain this, one has to determine accurately the ability of animal to withstand blood volume reduction by simple hemorrhage and by muscle trauma. The present investigation deals mainly with a comparative study of the mortality rates in the two groups of dogs. The results show that there are other factors in addition to the reduction in blood volume which contribute to the fatal outcome in traumatic shock.

**METHODS.** Simple hemorrhage and muscle trauma experiments were carried out on 30 and 40 healthy mongrels, respectively, ranging from 6 to 21 kgm. in body weight. On the day before each experiment, a control plasma volume and several hematocrits were taken. Thereafter, the animal was given only water ad libitum.

Plasma volume determinations were made with the dye (T-1824) dilution method (4). The serum dye concentration was determined by a König-Martens visual spectrophotometer, using a wave length of 620 m $\mu$ . The extrapolation of the time-concentration curve was made on a semilogarithmic plot (5). Relative changes in serum protein concentration were estimated from the changes in the refractive index of the serum with an Abbe refractometer (6). The individual protein value thus obtained checked within 0.25 gram per cent of the value obtained with the micro-Kjedahl method, and furthermore, there was close agreement between the relative changes in protein concentration by the two methods (7). The hematocrit values were measured by centrifuging heparinized blood samples in Wintrobe tubes at 3000 r.p.m. for 30 minutes. The total blood

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<sup>2</sup> A preliminary report of part of this work appeared in *Fed. Proc.* 4: 75, 1945.

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volume was calculated from the measured plasma volume and the average hematocrit value, without however correction for the small amount of plasma trapped between cells<sup>4</sup>. On the day of the hemorrhage experiment the animal was placed on its back in a cradle-like animal board. A control heart rate and rectal temperature were taken. Mean blood pressure was determined by needle puncture of the femoral artery. The animal was then given ether for 15 to 20 minutes<sup>5</sup>, during which time the femoral artery was exposed and cannulated, and bleeding begun; about three-fifths of the total blood volume to be withdrawn was removed at the beginning and nearly all the remaining portion removed at the end of the period of anesthesia. In a few animals, a small volume of blood not exceeding 10 per cent of the total volume bled had to be taken out or returned to the animal within half an hour following the massive hemorrhage, depending upon the condition of the animal. With the exception of a few preliminary experiments, the amount of blood removed was such that the residual blood volume would be around 55 to 60 cc. per kgm., not counting the compensatory dilution. The total volume varies from 32 to 47 cc. with an average of 40 cc. per kgm. After the hemorrhage, the femoral arterial cannula was connected to a mercury manometer. Determinations of mean blood pressure and heart rate were made every 20 minutes for a period of 6 hours. While the animal was recovering from etherization, the femoral vessels of the opposite side were exposed under 2 per cent procaine anesthesia. The blood volume was again determined one to two hours after the hemorrhage. The dye was injected into the exposed femoral vein and subsequent blood samples were withdrawn from the femoral artery. If the animal died within 6 hours, a gross autopsy was performed. In case the animal survived 6 hours, the wounds in the femoral region were closed and the dog was put into a cage without being given water for the duration of a 24 hour survival period. This is necessary, for if water were given, the blood volume would increase rapidly (10). By withholding water for 24 hours following hemorrhage, the blood volumes show little or no change overnight.

The detailed procedures for the muscle contusion in the trauma experiments have been reported elsewhere (11,12). The duration of the ether anesthesia varies from 10 to 30 minutes. The post-traumatic procedure was identical with that followed in the simple hemorrhage experiments, except that the femoral artery was not cannulated and the mean blood pressures were taken by repeated punctures of the exposed femoral artery. If the animal survived for 6 hours on the board, he was returned to the cage and water was given. In several of the 24 hour survivals, the blood volume was determined again on the day after trauma and was found to be not significantly different from that determined immediately following injury, despite the fact that the dogs invariably had consumed a considerable quantity of water in the interim.

<sup>4</sup> Root, Roughton and Gregersen (8,9) checked the dye (T-1824) dilution method with an improved CO method, and they found that the blood volumes in normal, hemorrhaged and traumatized animals obtained by the two independent methods are accurate within about 5 per cent.

<sup>5</sup> Ether anesthesia was given while the bleeding was performed, so that this series is comparable with the trauma experiments in this respect.

TABLE 1

*Data on dogs hemorrhaged under ether, including changes in blood volumes, hematocrit values and serum protein concentrations*

DOG NUMBER AND SEX	WEIGHT	CONTROL			AFTER HEMORRHAGE			CHANGE			FATE
		B. V.	Hct.	Protein	B. V.	Hct.	Protein	B. V. 100 × (1)-(4)	Hct. (5)-(2)	Protein (6)-(3)	
		(1)	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	
	kgm.	cc./kgm.	%	gm. %	cc./kgm.	%	gm. %	%	%	gm. %	
H 1 ♀	11.1	108	42.0	5.5	77	42.8	4.7	29	+0.8	-0.8	Survived
H 2 ♀	7.4	112	39.5	6.1	74	33.2	4.8	34	-6.3	-1.3	Died, 5 hr.
H 3 ♀	7.6	111	53.6	6.7	73	43.5	5.4	34	-10.1	-1.3	Survived
H 4 ♂	8.1	106	41.5	6.6	70	31.0	5.1	34	-10.5	-1.5	Survived
H 5 ♀	8.6	107	45.5	5.5	70	39.6	4.8	35	-5.9	-0.7	Survived
H 6 ♀	20.6	92	41.7	6.4	69	42.2	5.4	25	-0.5	-1.0	Survived
H 7 ♀	8.8	100	44.6	5.6	69	37.0	4.8	31	-7.6	-0.8	Survived
H 8 ♂	8.6	98	38.7	5.5	68	39.5	4.5	31	+0.8	-1.0	Survived
H 9 ♂	8.0	102	41.0	6.5	68	34.9	5.5	33	-6.1	-1.0	Died, 3.5 hr.
H 10 ♀	10.6	89	45.3	5.7	65	41.1	4.9	27	-4.2	-0.8	Survived
H 11 ♀	10.0	97	25.8	4.8	65	24.2	4.2	33	-1.6	-0.6	Survived
H 12 ♂	9.1	100	43.6	6.2	63	46.8	5.6	37	+3.2	-0.6	Died, 3 hr.
H 13 ♂	12.3	90	32.9	4.9	62	25.9	4.2	31	-7.0	-0.7	Survived
H 14 ♂	10.7	93	29.2	5.4	62	30.6	4.5	33	+1.4	-0.9	Survived
H 15 ♂	11.4	92	46.0	6.7	62	40.5	6.1	33	-5.5	-0.6	Survived
H 16 ♂	10.1	104	30.9	5.2	62	22.8	4.3	40	-8.1	-0.9	Died, 2.8 hr.
H 17 ♀	10.6	94	41.1	5.0	61	35.9	4.0	35	-5.2	-1.0	Survived
H 18 ♂	10.3	103	44.2	5.9	61	38.6	4.8	41	-5.6	-1.1	Died, 4 hr.
H 19 ♂	11.4	87	45.7	5.3	60	48.0	4.7	31	+2.3	-0.6	Survived
H 20 ♂	10.7	93	48.1	6.8	60	36.8	5.0	35	-11.3	-1.8	Survived
H 21 ♀	10.5	103	36.4	5.3	60	36.8	4.7	42	+0.4	-0.6	Survived
H 22 ♀	9.9	104	45.4	5.2	60	41.6	4.5	43	-3.8	-0.7	Survived
H 23 ♂	9.7	93	44.4	5.6	58	36.8	4.7	38	-7.6	-0.9	Survived
H 24 ♂	9.6	103	41.5	5.5	58	30.1	4.2	44	-11.4	-1.3	Died, 6+ hr.
H 25 ♂	10.9	90	41.2	?	56	37.2	?	38	-4.0	?	Died, 2 hr.
H 26 ♀	11.2	95	41.9	5.6	56	35.9	4.6	41	-6.0	-1.0	Died, 3 hr.
H 27 ♂	9.5	90	48.9	5.7	54	41.4	4.9	40	-7.5	-0.8	Died, 6+ hr.
H 28 ♀	15.8	102	51.9	6.3	54	40.7	5.2	47	-11.2	-1.1	Died, 4.5 hr.
H 29 ♀	9.8	92	45.9	5.9	53	40.1	4.8	42	-5.8	-1.1	Died, 6+ hr.
H 30 ♂	10.6	86	38.7	5.5	48	38.6	5.5	44	-0.1	0.0	Died, 2 hr.
Mean . . . .	10.4	97.9	41.9	5.8		37.1	4.9		-4.8	-0.9	
Standard error .		1.3	1.1	0.1					0.8	0.06	

RESULTS. Table 1 includes the data on blood volume determinations, hematocrit values and serum protein concentrations before and after hemorrhage. The data are arranged according to the residual blood volume, that is, the blood volume determined after hemorrhage and expressed in cubic centimeters per

kilogram of body weight. It is apparent that animals do not survive if the residual blood volume is less than 58 cc. per kgm. of body weight. Four of the five animals that died with a residual blood volume larger than 58 cc. per kgm. of body weight presented a different clinical picture from those which survived, the most striking feature being that their mean blood pressure was reduced below 50 mm. Hg at all times during the period of observation.

The data on the residual blood volume were analyzed according to a modification of the procedure suggested for determining the median lethal dose from the dosage-effect curve (13). The method may be exemplified with the data of table 1, in which the experiments arranged according to the magnitude of the

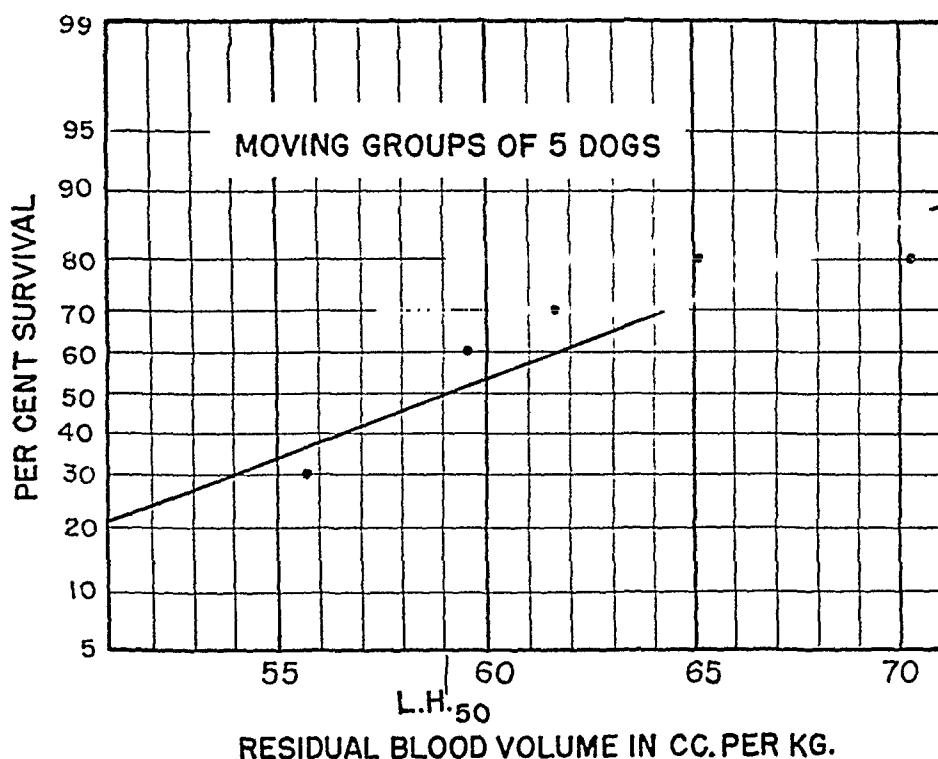


Fig. 1. Relationship between survival and residual blood volume of the hemorrhaged animals plotted on probability paper.

residual blood volume are subdivided into groups of 5 animals each. These animals were combined in overlapping groups and the average blood volume and percentage survival determined. Thus for the first two groups of 5 the average residual blood volume is 70.3 cc. per kgm. and 80 per cent (8 out of 10) survived, for the second and third group of the average blood volume is 65.2 cc. per kgm. and 80 per cent survived, and so on. The percentage survival was then plotted against the corresponding average residual blood volume on probability paper as shown in figure 1, and the best straight line drawn by eye through the points. The residual blood volume at 50 per cent survival (L. H.<sub>50</sub>) is read as 59.1 cc. per kgm. of body weight<sup>6</sup>. The standard error of the

<sup>6</sup> The validity of the method of analysis has been tested by grouping the animals differently, for instance, 4 or 6 dogs in each group instead of 5 dogs, and the L.H.<sub>50</sub> value thus obtained is not appreciably different.

TABLE 2

*Data on traumatized dogs, including changes in blood volumes, hematocrit values and serum protein concentrations*

DOG NUMBER AND SEX	WEIGHT	CONTROL			AFTER TRAUMA			CHANGE			FATE
		B. V.	Hct.	Protein	B. V.	Hct.	Protein	B. V.	Hct.	Protein	
	kgm.	cc./kgm.	%	gm. %	cc./kgm.	%	gm. %	%	%	gm. %	
T 1 ♀	13.8	109	43.5	6.5	93	57.3	6.7	15	+13.8	+0.2	Survived
T 2 ♂	10.6	102	43.7	5.3	91	61.0	5.6	11	+17.3	+0.3	Survived
T 3 ♂	12.8	110	46.3	7.3	85	45.6	7.3	23	-0.7	0.0	Survived
T 4 ♀	8.8	113	43.4	5.8	83	49.5	5.8	27	+6.1	0.0	Survived
T 5 ♂	11.7	110	39.9	5.8	82	47.2	6.4	26	+7.3	+0.6	Died, 4.7 hr.
T 6 ♂	9.0	117	42.4	5.0	80	48.0	5.2	32	+5.6	+0.2	Survived
T 7 ♀	8.5	93	38.4	5.9	80	54.8	6.3	14	+16.4	+0.4	Died, 5.1 hr.
T 8 ♀	11.1	111	49.4	6.5	79	53.1	6.5	29	+3.7	0.0	Survived
T 9 ♂	10.9	108	52.4	5.8	78	62.0	6.3	28	+9.6	+0.5	Died, 6 hr.
T 10 ♀	8.3	105	51.8	5.6	75	52.8	5.4	29	+1.0	-0.2	Survived
T 11 ♀	10.0	97	35.6	4.9	73	39.3	4.9	25	+3.7	0.0	Survived
T 12 ♀	10.5	106	40.1	5.0	73	49.5	5.2	31	+9.4	+0.2	Died, 3.2 hr.
T 13 ♂	13.9	92	45.0	5.5	72	51.4	5.9	22	+6.4	+0.4	Survived
T 14 ♀	14.6	101	46.3	?	72	53.5	?	29	+7.2	?	Survived
T 15 ♀	8.5	94	31.2	4.8	71	35.3	4.5	24	+4.1	-0.3	Survived
T 16 ♀	12.6	98	47.3	5.7	70	47.1	5.7	29	-0.2	0.0	Survived
T 17 ♂	9.3	100	37.2	6.9	70	39.7	6.6	30	+2.5	-0.3	Died, 6 hr.
T 18 ♀	8.6	104	41.0	5.2	70	46.3	5.2	33	+5.3	0.0	Died, 5 hr.
T 19 ♀	9.3	104	47.3	5.6	70	48.5	5.6	33	+1.2	0.0	Died, 2.7 hr.
T 20 ♀	6.4	120	45.9	4.9	69	42.4	4.4	43	-3.5	-0.5	Died, 3.8 hr.
T 21 ♀	5.5	106	38.0	4.9	69	34.8	4.3	35	-3.2	-0.6	Died, 4.1 hr.
T 22 ♂	10.9	101	43.8	5.5	68	46.7	5.6	33	+2.9	+0.1	Survived
T 23 ♀	12.1	95	42.7	5.4	68	51.9	5.5	28	+9.2	+0.1	Died, 4.2 hr.
T 24 ♀	8.5	106	47.3	5.7	68	43.9	5.2	36	-3.4	-0.5	Died, 2 hr.
T 25 ♂	8.8	102	38.4	5.5	67	50.0	5.8	34	+11.6	+0.3	Died, 6+ hr.
T 26 ♂	9.0	113	38.6	6.3	67	39.0	6.3	41	+0.4	0.0	Died, 2.5 hr.
T 27 ♂	11.1	91	42.7	5.2	65	47.2	5.2	29	+4.5	0.0	Survived
T 28 ♂	9.3	97	45.0	5.5	65	48.8	6.2	33	+3.8	+0.7	Survived
T 29 ♀	8.9	117	35.3	5.2	65	40.2	5.0	44	+4.9	-0.2	Died, 3 hr.
T 30 ♀	11.2	101	51.0	7.8	64	44.6	6.6	37	-6.4	-1.2	Died, 2 hr.
T 31 ♀	11.7	93	38.2	5.5	62	51.9	6.3	33	+13.7	+0.8	Died, 4.2 hr.
T 32 ♂	8.3	108	33.0	5.9	62	40.0	5.9	43	+7.0	0.0	Died, 4.2 hr.
T 33 ♀	8.1	89	37.6	4.7	60	36.0	4.7	33	-1.6	0.0	Died, 3.1 hr.
T 34 ♀	8.1	93	44.6	5.9	59	41.3	5.3	37	-3.3	-0.6	Died, 2.1 hr.
T 35 ♀	14.9	78	35.0	6.3	58	42.7	6.3	26	+7.7	0.0	Died, 6+ hr.

TABLE 2—*Concluded*

DOG NUMBER AND SEX	WEIGHT	CONTROL			AFTER TRAUMA			CHANGE			FATE
		B. V.	Hct.	Protein	B. V.	Hct.	Pro- tein	B. V.	Hct.	Protein	
	kgm.	cc./kgm.	%	gm. %	cc./ kgm.	%	gm. %	%	%	gm. %	
T 36 ♀	8.5	88	53.0	6.2	58	55.3	6.0	34	+2.3	-0.2	Died, 4 hr.
T 37 ♂	14.4	83	40.8	6.5	57	50.0	6.9	31	+9.2	+0.4	Died, 2.4 hr.
T 38 ♀	10.4	92	47.5	5.8	57	44.8	5.1	38	-2.7	-0.7	Died, 6+ hr.
T 39 ♂	10.1	85	48.1	6.0	56	51.1	6.0	34	+3.0	0.0	Died, 6 hr.
T 40 ♀	10.2	96	48.2	7.1	56	42.8	6.3	42	-5.4	-0.8	Died, 6+ hr.
Mean....	10.2	100.7	42.9	5.8		47.0	5.8		+4.1	0.0	
Standard error...		1.5	0.9	0.1					0.9	0.07	

L.  $H_{.50}$  is 2.9 cc. per kgm. obtained as follows: the residual blood volume at 84 per cent survival is read as 69.1 cc. per kgm., or 10 cc. per kgm. larger than L.  $H_{.50}$ ; the number of animals in table 1 within 10 cc. per kgm. of L.  $H_{.50}$  is 24; then the standard error may be computed approximately as  $10 / \sqrt{24/2} = 2.9$ .

If there are no other factors affecting the death of traumatized dogs, then we should find that the relation between mortality and residual blood volume will be comparable to that of the hemorrhaged dogs. The data on 40 muscle trauma experiments are summarized in table 2. It is clear at once that traumatized dogs die with a much higher residual blood volume than the hemorrhaged animals. Indeed, after muscle trauma animals seldom survived with a residual blood volume of less than 70 cc. per kgm. of body weight. It should be noted that the control blood volumes in the two groups of animals are not significantly different ( $97.9 \pm 1.3$  cc. per kgm. in the hemorrhage series vs  $100.7 \pm 1.5$  cc. per kgm. in the muscle trauma series). By applying the same method of analysis, the residual blood volume at 50 per cent survival (L.  $H_{.50}$ ) in the muscle trauma series is  $73.4 \pm 3.0$  cc. per kgm. of body weight. The difference of 14.3 cc. per kgm. between this value and the L.  $H_{.50}$  of the hemorrhage series is 3.4 times its standard error. Thus the odds are very remote that the difference is due merely to chance.

If, instead of comparing the 50 per cent survival points, we compare the percentage of survivals at a definite residual blood volume, for instance, 66 cc. per kgm. of body weight (midway between the two L.  $H_{.50}$  values), we find that in the hemorrhage series the survival is  $76 \pm 8.7$  per cent, whereas in the muscle trauma series the survival is  $25 \pm 8.3$  per cent. The difference between these two percentages has about the same degree of statistical significance as that between the L.  $H_{.50}$  values.

There are other differences in these experiments such as the changes in serum protein concentrations and the hematocrit values (tables 1 and 2). In the hemor-

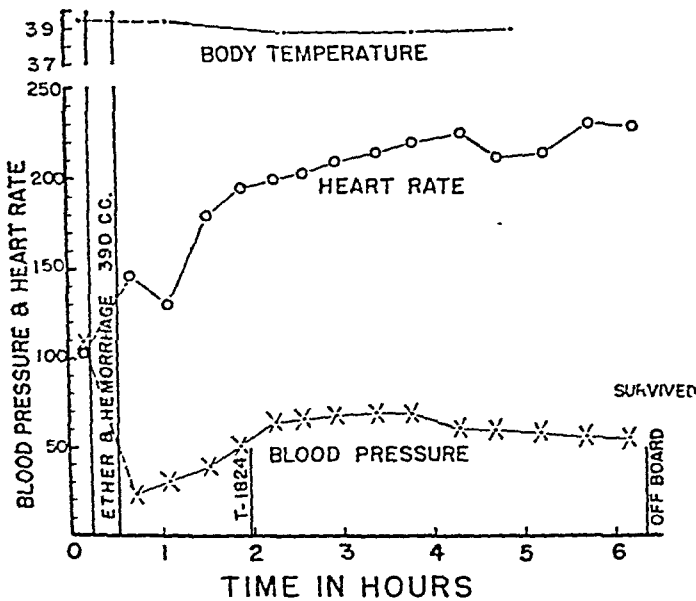


rhage series the decrease in serum protein is from 5.8 to 4.9 grams per cent ( $-0.9 \pm 0.06$  per cent) and in hematocrit is from 41.9 to 37.1 per cent ( $-4.8 \pm 0.8$  per cent). In the muscle trauma series, however, the serum protein does not show any change and the hematocrit shows an increase from 42.9 to 47.0 per cent ( $+4.1 \pm 0.9$  per cent). The differences between the corresponding values in the two series are statistically significant, whereas the corresponding control values are comparable.

## HEMORRHAGE

NORMAL DOG H 13  
WEIGHT 12.3 KG.M.

	BEFORE	AFTER
BLOOD VOLUME, cc/KGM.	90	62
HEMATOCRIT, %	33	26
SERUM PROTEIN, gm. %	4.9	4.2



## TRAUMA

NORMAL DOG T 31  
WEIGHT 10.7 KG.M.

	BEFORE	AFTER
BLOOD VOLUME, cc/KGM.	93	62
HEMATOCRIT, %	38	52
SERUM PROTEIN, gm. %	5.5	6.3

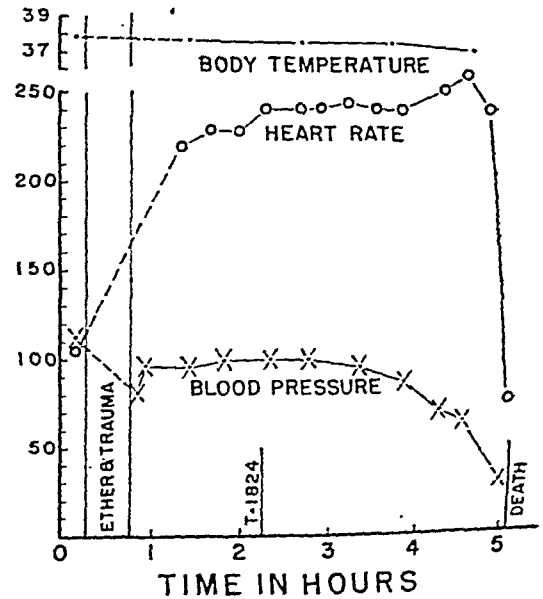


Fig. 2. Comparison of the clinical manifestations (body temperature, pulse rate and mean blood pressure) in a typical hemorrhaged animal (dog H 13) and a typical traumatized animal (dog T 31), having the same residual blood volume. Note that the hemorrhaged animal, despite its low blood pressure, survived whereas the traumatized animal died after 4 hours.

The clinical charts of a typical hemorrhage and a typical trauma experiment are presented in figure 2. It is to be noted especially that the control blood volumes and residual blood volumes are the same in these two experiments and yet the hemorrhaged dog survived, whereas the traumatized dog died. The control blood pressures and heart rates were also the same. The hemorrhaged animal had a very low mean blood pressure (22 mm. Hg) immediately after bleeding which climbed slowly to a level of 70 mm. Hg; also it took almost two hours for the heart rate to exceed 200 beats per minute. On the other hand, after injury the traumatized dog initially had a high mean blood pressure which was maintained for 3 to 4 hours and immediate tachycardia of 220 beats per minute. Nevertheless, this animal succumbed suddenly 4 hours after trauma.

DISCUSSION. In recent years traumatic shock has come to be regarded as largely the result of a loss of circulating fluid into the injured tissues. In 1930, Blalock (2) reported a series of 8 dogs in which one leg was traumatized under barbitol anesthesia. At the end of 1 to 6 hours the animals were sacrificed and the traumatized and non-traumatized legs were amputated and weighed. The difference in weight of the two legs which accounts for the fluid and blood loss varied from 4.2 to 8.0 per cent of body weight (average, 5.1 per cent). In a more or less similar manner, Parsons and Phemister (3) traumatized the legs of 6 dogs with a loss of fluid into the injured area varying from 4.2 to 6.0 per cent of body weight (average, 5.3 per cent). However, it cannot be maintained that all or even the majority of instances of traumatic shock seen in surgical practice are accompanied by a sufficiently large fluid loss to cause death. In some cases profound shock is associated with trivial injuries. That, therefore, animals in traumatic shock may succumb with a negligible loss of circulating volume is a common experience to all investigators. Our quantitative data serve only to substantiate this impression.

It is important to ascertain that residual blood volume remains unchanged during the post-injury period of observation. It is known that following injury compensatory fluid shifts occur and according to Adolph, Gerbasi and Lepore (14), the major shift after hemorrhage requires about 22 minutes for approximate completion. Our determination of the residual blood volume was carried out one to two hours following hemorrhage when the process of plasma dilution is virtually complete. During the 6 hours of observation repeated determinations of hematocrit and serum protein showed no progressive changes. Furthermore, the total blood volume in 5 dogs that survived hemorrhage showed an average increase in blood volume determined 24 hours later of only 3 per cent (provided no water was given overnight). In the case of muscle trauma, in addition to fluid shifts, leakage of blood and fluid into the injured areas continues for some time after one to two hours, but the blood volume remains fairly constant throughout the period of observation (12), indicating either that leakage is small or that leakage is adequately compensated by the concurrent fluid shift into the plasma compartment (15).

The analysis of the mortality rate in the two series was based on the residual blood volume in cubic centimeters per kilograms of body weight rather than upon the percentage reduction in blood volume from the control (or upon the percentage that the residual formed of the control volume). The former seemed more convenient. Further, the control levels do not vary greatly nor does the residual blood volume depend very much on the control value. Thus both methods of analysis should yield essentially equivalent results. In fact, in terms of percent reduction in blood volume, the value accompanied by 50 per cent survival is  $37.4 \pm 1.7$  per cent for the hemorrhage series and  $30.4 \pm 2.3$  per cent for the trauma series. The difference is statistically significant although not quite to the same extent as when residual blood volume was used directly. *There is no doubt that the ability of the hemorrhaged dogs to withstand a critical quantity of blood loss is strikingly greater than that of dogs receiving muscle trauma.*

Since animals that receive severe insult either from simple hemorrhage or muscle trauma will necessarily manifest a high heart rate and low blood pressure, it is meaningful to compare these clinical signs only in animals of the two series having approximately the same residual blood volume. Comparisons of heart rate and blood pressure are therefore made only on the animals having a residual blood volume between 60 to 70 cc. per kgm. of body weight. Among the 19 hemorrhaged animals in this group 4 died; whereas in the trauma series 13 of the 18 animals in this group died. This is of course merely a restatement of the relation of mortality to residual blood volume already discussed. The clinical signs shown by the animals that succumbed in shock are different from those of the survivals in the same series. Any statistical analysis of the clinical data for all the animals in this range of residual blood volumes is therefore difficult. We may however make the following generalizations. Most of the traumatized animals exhibited a high heart rate (usually exceeding 200 beats per min.) immediately following the insult, whereas the animals subjected to hemorrhage reached such levels of heart rate only after a period of time. Hemorrhaged animals survive tremendous reductions of the mean blood pressure, sometimes as low as 22 mm. Hg during the first half hour after injury. Traumatized dogs, on the other hand, seldom if ever survived when the mean blood pressure was reduced below 64 mm. Hg in the first half hour after trauma. In a large series of over 100 muscle trauma experiments accumulated in this laboratory only three dogs survived when the mean blood pressure fell slightly below 70 mm. Hg within the first half hour after muscle trauma. Furthermore, the traumatized dogs have a higher mean blood pressure during impending shock than the hemorrhaged animals with the same residual blood volume. Death of the traumatized dog is usually preceded by a rapid decline of the pressure. Indeed, it is our common experience that the traumatized animals having a mean blood pressure over 60 mm. Hg died suddenly within half an hour, whereas hemorrhaged animals survived and walked around with a mean blood pressure of 50 mm. Hg after 6 hours of observation on the animal board. Equally impressive is the fact that in traumatized animals evidences of central nervous depression are prominent, and such signs appear early when the mean blood pressure level is still high. Many times functional decerebrate rigidity appeared when the mean blood pressure was as high as 60 mm. Hg, whereas hemorrhaged animals are not as depressed and show no decerebrate rigidity even at pressures of 30 mm. Hg.

The two experiments also show significant differences in other respects. The marked decreases in the serum protein concentrations and in the hematocrit levels in the hemorrhage series indicate that a considerable volume of fluid must have entered into the circulatory system as a result of the low intravascular hydrostatic pressure following the hemorrhage. In the muscle trauma series, such a shift is not clearly evident, for the reasons that the shift is probably small and that the effect is in part masked by a concurrent leakage of fluid into the injured areas (15, 16). Also in deafferented animals following muscle trauma there is a slight decrease of serum protein concentrations and only a slight increase in hematocrit values (17) which indicates that the afferent impulses from the injured regions in the traumatized normal animals played an important rôle not only in vasoconstriction and high systemic arterial pressure but also in an

increased contraction of the spleen. The latter will result in high hematocrit values which will further increase the apparent viscosity of the blood (18, 19), and contribute to hypoxia of the tissues.

In agreement with the above evidence indicating other factors besides the blood volume reduction in causing the mortality rate in traumatic shock, Root, Walcott and Gregersen (20) have found that the calculated total peripheral resistance in traumatized dogs is greater than that shown by dogs in which the blood volume has been reduced to the same extent by hemorrhage. The fluorescein circulation time in the traumatized animals is also considerably longer than that in the hemorrhaged animals (21).

Other investigations of the differences between hemorrhaged and traumatized dogs have been carried out in this laboratory. Thus, Overman and Wang (22) and Wang (17) have studied the rôle of afferent stimulation and the effect of deafferentation upon the clinical signs and mortality rates of the shocked animals.

#### SUMMARY AND CONCLUSIONS

Our quantitative data show that in the hemorrhage series the residual blood volume at 50 per cent mortality ( $L. H_{.50}$ ) is  $59.1 \pm 2.9$  cc. per kgm. of body weight, whereas in the trauma series the  $L. H_{.50}$  is  $73.4 \pm 3.0$  cc. per kgm. of body weight. The difference between the  $L. H_{.50}$  values for these two groups is statistically significant. This indicates that the loss of the circulating blood volume alone is not adequate to explain the high mortality rate in traumatized dogs.

The traumatized animals as compared with hemorrhaged animals have early tachycardia exceeding 200 beats per minute, high mean blood pressure, high hematocrit values, early depression of the central nervous system, and according to other investigations high calculated peripheral resistance (20) and high fluorescein circulation time (21).

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# THE EFFECTS OF ASPHYXIATION AND NARCOSIS ON PERIPHERAL NERVE POLARIZATION AND CONDUCTION

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A decrease in rest current accompanying the loss of excitability in rabbit nerve deprived of its blood supply was observed by Koch (5). Gerard (4) recorded the development of a potential difference between an area of frog nerve in nitrogen and an area in oxygen. Both Furusawa (3) and Cowan (1) noted the decrease of the demarcation potential of asphyxiated crab nerves. Depolarization of the spinal cord during asphyxiation and anoxia has been demonstrated recently by van Harreveld (8). These data indicate that the primary effect of oxygen lack on the nerve consists in depolarization of the membranes of the fibers, and, on the basis of the membrane theory of conduction, when such depolarization is carried far enough, propagation of the impulse becomes impossible. The propagated spike also disappears in the presence of CO (6), CN (7), and alcohol (2). It is not known whether the arrest of conduction caused by these substances is also the result of depolarization or that conduction in the still polarized fiber is barred directly. A decrease in the rest current of frog nerve narcotized with ether vapor has been observed (5). The present work was undertaken to investigate the effect of nitrogen, alcohol, ether, cyanide and carbon monoxide on the resting potential and action potential simultaneously, and to ascertain whether depression of the propagated spike and depolarization always occur together.

**METHODS.** The peroneal and tibial nerves of cats and rabbits, some bullfrog sciatic and marine crustacean (*Panulirus interruptus*) nerves were used. After removal from the animal the mammalian nerve was placed in Tyrode solution, frog nerve in Ringer and the lobster nerves in sea water all for about ten minutes. The nerve was then placed in the trough, *A*, figure 1, of the chamber described in detail previously (9). Six silver-silver chloride electrodes contacted the nerve in the trough. A small partition, *B*, of  $\frac{1}{8}$  inch thick lucite had been added which was sealed with vaseline into the chamber, dividing it into two sections. Unless otherwise designated the partition was always placed between electrodes 1 and 2 leaving five leads in the larger section and one lead in the smaller section of the chamber.

There were three gas inlets in the chamber, two of them in the large, and one in the small section. Of the two in the large section, one, *C*, was used only for oxygen and nitrogen, and the other, *D*, for CO, HCN, alcohol and ether. Contamination of the oxygen system was prevented in this way. Each section of the chamber possessed one gas outlet. At the start of an experiment, one of the two inlets in the large section was closed, the chamber placed in a constant

temperature bath, and oxygen admitted through the other inlets so that the whole nerve was well oxygenated in both sections. After about fifteen minutes, the oxygen in the large section was replaced by the gas to be investigated. Oxygen was continuously blown into the small section throughout an experiment to keep this area of the nerve normal. Then any electrical changes in the nerve area in the large section could be compared directly with the electric properties of the normal nerve area in oxygen. Commercial nitrogen, which is pure enough for mammalian nerve asphyxiation, was passed over heated copper gauze when used for frog nerve. Commercial carbon monoxide of 100 per cent concentration, and 10 per

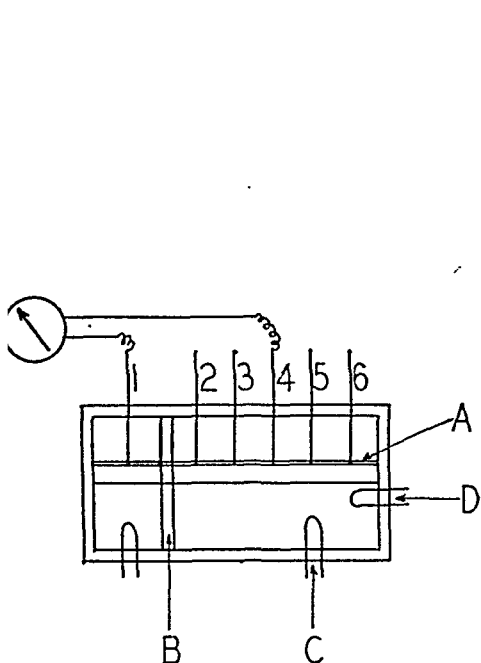


Fig. 1

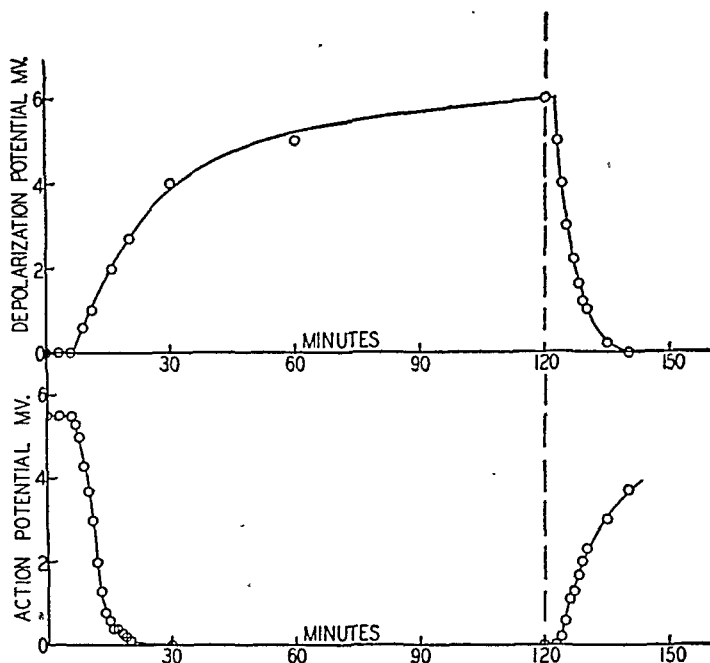


Fig. 2

Fig. 1. Diagram of the lucite nerve chamber showing the partition, B, and the additional leads, 1 and 4, connecting with the galvanometer (see text). The separate gas inlets C for  $N_2$  and  $O_2$  and D for narcotics, HCN, and CO are also shown.

Fig. 2. Upper curve shows development of the depolarization potential (mV) in cat peroneal asphyxiated in  $N_2$  for 2 hours. Asphyxiation starts at time 0 and dashed line indicates time  $O_2$  replaces  $N_2$  for recovery. Lower curve shows simultaneous recording of the action potential (mV).

cent HCN in air were used. The alcohol vapor employed was obtained by bubbling oxygen slowly through 10 to 15 per cent alcohol in distilled water. The ether vapor was acquired by passing oxygen through a flask containing a small amount of ether and this was further diluted in the chamber by continuing to admit oxygen through the other inlet in this section. No measurement was made of the concentrations of the alcohol and ether vapors, but these mixtures were found experimentally to depress and eventually extinguish the action potential in about the same time as asphyxiation.

Of the six leads, number 1 and number 4 in figure 1, one on either side of the partition, were connected with a very sensitive direct-current amplifier designed

by R. C. Hawes of the National Technical Laboratories, South Pasadena (see appendix (8)). The amplified potential changes occurring between these electrodes were recorded by a galvanometer. The other four leads 2, 3, 5 and 6, were connected as follows: one pair, 5 and 6, to a thyatron square wave generator, and the other pair, 2 and 3, to a cathode ray oscilloscope. These two pairs could be interchanged as stimulating or recording electrodes by a switch in the circuit.

**RESULTS. Nitrogen.** Sixty rabbit nerves, twenty-five cat nerves and six frog nerves were exposed to nitrogen in one section and oxygen in the other. The simultaneous recordings of the A.P. and D.P. are plotted in figure 2. These data are from a cat peroneal. No changes occurred for 3 to 8 minutes in rabbit nerve, 5 to 10 minutes in cat nerve and 60 to 80 minutes in frog nerve. Then the development of a potential across the partition was recorded, the nerve area in nitrogen becoming negative to that in oxygen. This potential will hereafter be referred to as the depolarization potential, or D.P. Simultaneously with the onset of the D.P., the action potential, A.P., began decreasing in size. In the next few minutes, with mammalian nerve, the A.P. decreased more rapidly usually disappearing when the D.P. had reached  $\frac{1}{2}$  to  $\frac{2}{3}$  of its final maximum value. After the readmission of oxygen in place of the nitrogen there is a brief "latent period" of 1-3 minutes with rabbit, cat, as well as frog nerve, in which no change occurs, then the D.P. decreases rapidly accompanied by an equally rapid recovery of the A.P. A very definite negative correlation between the A.P. and D.P. exists. In figure 2 the A.P. vanished after 30 minutes in nitrogen when the D.P. reached 4 mV. At the end of two hours the D.P. is 6 mV. The A.P. begins to reappear in oxygen only when the D.P. has decreased to 4 Vm. In recovering the D.P. disappears and the A.P. attains the original amplitude in five to ten minutes.

Since the fluid in the trough around the nerve causes "short-circuiting" both A.P. and D.P. are recorded considerably smaller than they actually are. Some of the thinner nerves which were carefully "blotted" to remove all excess moisture, developed depolarization potentials of 20-25 mV and gave action potentials in excess of 20 mV.

The threshold remained relatively constant in nitrogen until the rapid rise at the time of A.P. extinction. The threshold recovery also was very quick (see 9).

Many mammalian nerves were subjected to repeated asphyxiations with 10 minute intervals during which oxygen was supplied. As has been described previously (9), with a short oxygen period the following asphyxiation causes more rapid extinction of the A.P. It was found that the more rapid extinction of the A.P. was accompanied by a more rapid rise of the D.P. For example, the A.P. of a rabbit tibial during a first asphyxiation disappeared after 20 minutes in  $N_2$  at which time the D.P. was 5 mV. In the second asphyxiation the A.P. vanished after 11 minutes at which time the D.P. was 4.7 mV. In the first asphyxiation after 11 minutes the A.P. was  $\frac{2}{3}$  original size and the D.P. 2.5 mV.

*Cyanide.* Six rabbit nerves were exposed to a 10 per cent mixture of HCN in air. The effect begins in 2 to 5 minutes and the extinction time varies from 15 to 30 minutes. The results from data of a rabbit tibial are given in figure 3A. It is evident that the D.P. develops and the A.P. decreases as rapidly in the presence of 10 per cent cyanide as in the nitrogen experiments. The reversal in oxygen of these changes is also quick and the negative correlation

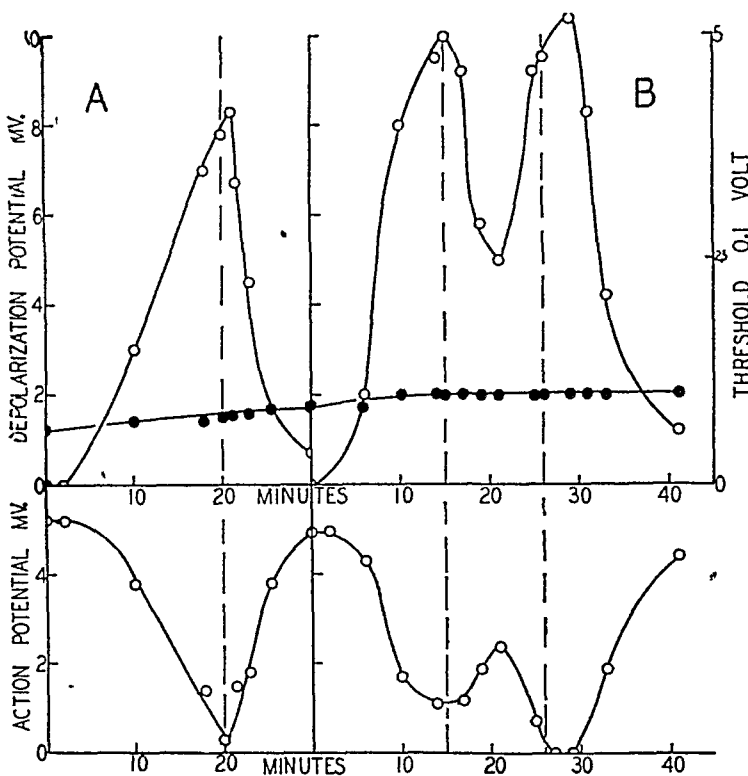


Fig. 3

Fig. 3A. Upper curve, open circles, illustrates D.P. development in a rabbit tibial in HCN. Curve with solid circles shows threshold change in HCN in 0.1 volt using right ordinate. Lower curve indicates A.P. change in same experiment. The HCN was admitted to the chamber at time 0, and replaced by O<sub>2</sub> (dashed line) after 20 minutes.

3B. Same connotation for these and all subsequent curves as in 3A. Curves illustrate changes in D.P., A.P. and threshold of rabbit tibial first in HCN for 15 minutes, then N<sub>2</sub> (first dashed line) for 11 minutes then oxygen (second dashed line).

Fig. 4. D.P., A.P. and threshold changes during an 8 minute exposure of rabbit peroneal to CO. Dashed line indicates when O<sub>2</sub> replaced CO.

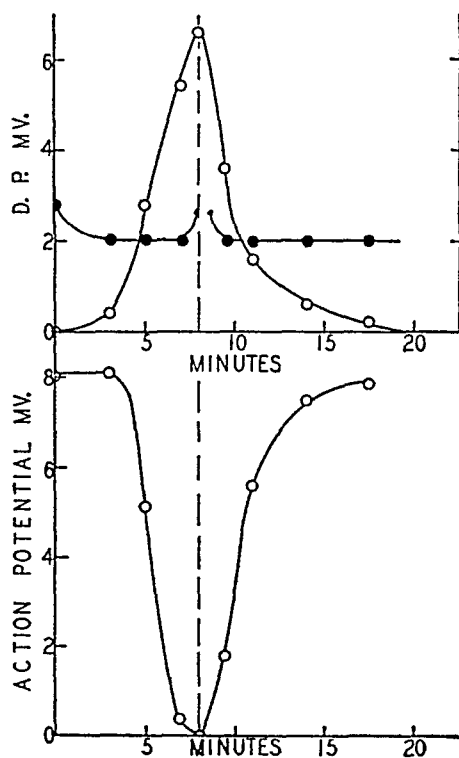


Fig. 4

of values is consistently evident. The threshold remains constant throughout cyanide exposure.

The medium surrounding the nerve in these experiments contained at least 18 per cent oxygen, more than enough for normal activity, yet depolarization and A.P. extinction occur. If the CN is removed by N<sub>2</sub> instead of O<sub>2</sub>, the result shown in figure 3B is obtained. The dashed line crossing the abscissa at 15 minutes indicates when N<sub>2</sub> replaced the HCN. Almost immediately following the admission of N<sub>2</sub> into chamber the D.P. decreases and the A.P. increases. This effect lasts only a few minutes after which asphyxiation by the N<sub>2</sub> occurs.



The threshold never changes during the experiment. With  $O_2$  replacing the  $N_2$ , second dashed line crossing the abscissa at 26 minutes, recovery takes place rapidly and is complete in 10 to 15 minutes.

*Carbon monoxide.* Sixteen rabbit, three cat and two frog nerves were subjected to 100 per cent carbon monoxide. The D.P. rises and the A.P. declines rapidly, extinction of the latter occurring in 6 to 12 minutes in rabbit nerves, 10–15 minutes in cat nerves. A typical simultaneous recording of A.P., D.P. and threshold is illustrated in figure 4. The activity in bullfrog nerves lasted 4 to 5 hours in CO. The recovery in oxygen is quick. The negative correlation

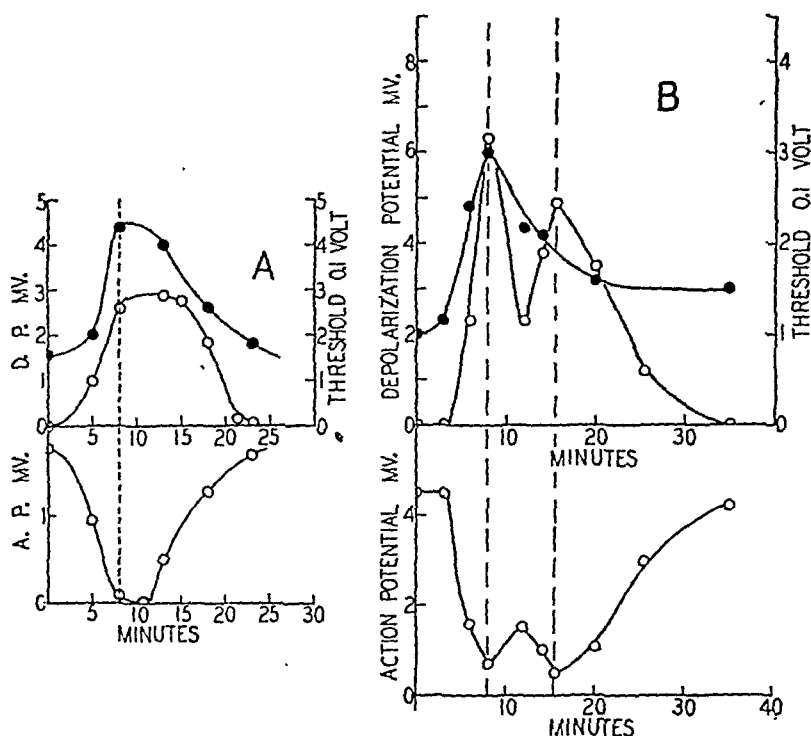


Fig. 5A. D.P., A.P. and threshold changes in rabbit tibial narcotized for 8 minutes in alcohol vapor.  $O_2$  replaced alcohol vapor at dashed line.

5B. D.P., A.P. and threshold changes of rabbit tibial narcotized 8 minutes in alcohol vapor, then exposed to  $N_2$  (first dashed line) and finally to  $O_2$  after 16 minutes (second dashed line).

between A.P. and D.P. values is always observed. The threshold remained constant until just before extinction when it rose rapidly. It decreased immediately to the original value with the oxygen recovery.

In CO asphyxiation temporary recoveries were not obtained when nitrogen replaced the CO after extinction of all activity.

*Alcohol.* Fifteen rabbit nerves and 4 cat nerves were subjected to alcohol vapor in the same manner as in nitrogen experiments. The effects on a rabbit tibial are given in figure 5A. The A.P. starts to decline and the D.P. starts to develop after 2 to 5 minutes. Extinction of the A.P. takes place in 11 minutes at which time the D.P. is 3 mV. The action potentials of rabbit nerves dis-

appeared in 10–15 minutes, of cat nerves in 20 to 30 minutes in the alcohol vapor. The recoveries in oxygen were not as rapid or as consistently complete as in anoxia. Usually the A.P. regained the original size, but in many instances the D.P. decreased only 30 to 40 percent. Thus the negative correlation between the two potentials, though evident in many experiments, was not always observed as it was in experiments in nitrogen, CO and CN. The threshold of excitability, black dot curve, rises slowly during narcosis and declines slowly during recovery. This is markedly different from the sudden rapid changes recorded in asphyxiation.

When nitrogen instead of oxygen is admitted to the chamber following narcosis, the alcohol vapor is removed and no oxygen is supplied. Yet the D.P. decreases,

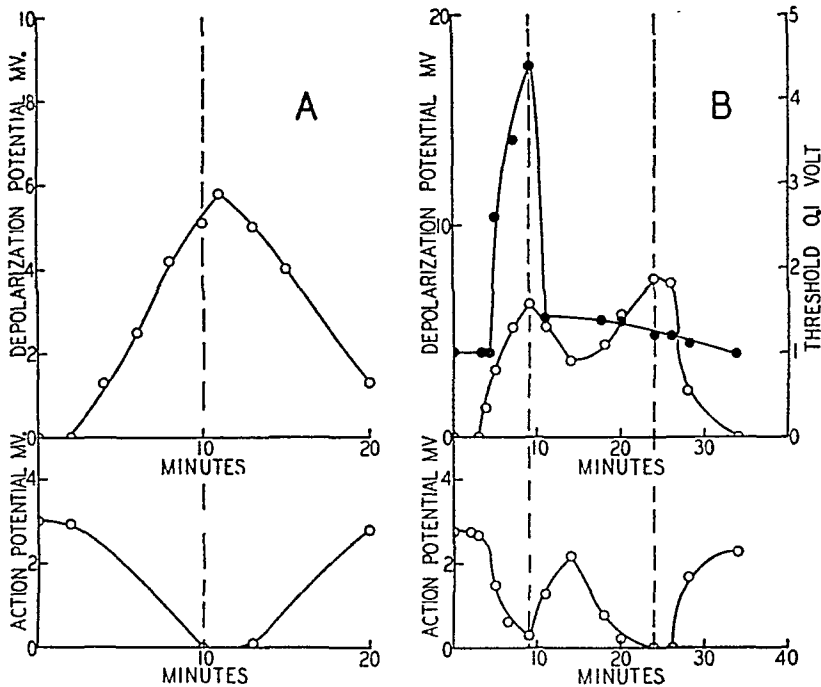


Fig. 6A. D.P., A.P. and threshold changes during narcosis of rabbit peroneal by ether vapor. O<sub>2</sub> replaced ether after 10 minutes (dashed line).

6B. D.P., A.P., and threshold changes in rabbit tibial first exposed to ether vapor for 9 minutes, then N<sub>2</sub> (dashed line) for 15 minutes, then oxygen (second dashed line).

the A.P. increases and the threshold declines as shown in figure 5B. These D.P. and A.P. recoveries are only temporary, lasting until asphyxiation due to the nitrogen takes effect. The threshold continues to be reduced in nitrogen towards the original value until the sudden rise at the time of A.P. extinction. Finally, the recovery of the A.P. and disappearance of the D.P. with oxygen after the nitrogen is the same as that already described for anoxia.

*Ether.* Nineteen rabbit and eight cat nerves were exposed to ether vapor (fig. 6A). The narcosis takes effect after 2 to 5 minutes after which the D.P. development and the A.P. decline go along together. The extinction time and the total D.P. developed were found to depend on the concentration of the ether. Low concentrations allowed extinction times of 10 to 20 minutes. High

concentrations extinguished all activity in 3 to 4 minutes and during this time very little if any depolarization is observed. Furthermore with high concentrations the reaction becomes irreversible after 5 minutes from the start of narcosis. The recovery in oxygen from low concentrations is slow and in some cases the correlation of the changes in A.P. and D.P. is absent.

Nitrogen following the dilute ether vapor causes temporary recoveries as shown in figure 6B. These recoveries were even observed in experiments in which the ether, first administered, was mixed with nitrogen instead of oxygen.

The threshold of excitability rises slowly in ether narcosis and decreases during recovery in oxygen (or nitrogen) as found with alcohol.

Since the amount of depolarization was somewhat variable in ether narcosis and, as seen in figure 6B, the D.P. in nitrogen excelled that recorded in ether, several nerves were first asphyxiated until the A.P. was gone and the D.P. maximum. Then ether vapor in oxygen was admitted to the chamber. If the D.P. were to be less in the ether than in the nitrogen a decline of the D.P. would be expected. This was not found. In other experiments nerves were left in concentrated ether vapor for 25 minutes. Although little depolarization had taken place in the first five minutes (reversible period), it did develop considerably in 10 minutes or more.

*Effect of anoxia on the action potential.* It is not known whether the decrease in the size of the action potential in asphyxiated nerve is caused by blocking of the conduction, decrease in the spike of the individual fiber or a combination of both effects (4, 9). Experiments were devised to investigate the reason for the A.P. decline during anoxia. The partition was placed between electrodes 3 and 4, (fig. 1) separating the stimulating and recording leads as well as the D.P. recording electrodes. Nitrogen was admitted to the section with leads 4, 5 and 6, oxygen to the other section. As asphyxiation took effect the nerve was stimulated maximally in oxygen and the A.P. recorded in nitrogen, and then stimulated maximally in nitrogen and the A.P. led off in oxygen. With this technique, if blocking is the cause of the A.P. diminution, then the decrease should be about the same in both gases. If the A.P. of the fibers actually grows smaller, then the deflection recorded in nitrogen should be smaller than that registered in oxygen. The results from two rabbit, two cat, and two frog nerves are listed in table 1. In column three the change of the action potential in the oxygenated part of the nerve is given in percent. Column four lists the percent change of the A.P. in the nitrogen section. The D.P. is listed in column six.

Almost always the action potential in nitrogen has decreased more than the potential in oxygen. This is seen clearly in column five in which the ratio of A.P. in nitrogen to A.P. in oxygen is listed. The decrease in oxygen indicates the occurrence of blocking, but the greater decrease in nitrogen means that the action potential size of the individual fibers has been reduced. The same effect has been found in alcohol experiments (2).

To check this result, further experiments were made using small bundles prepared from lobster nerves and containing two or three fibers. These fibers were

TABLE 1

*Per cent reduction in the size of nerve action potentials led off from the oxygen section of the chamber (column 3) and nitrogen section (column 4) during asphyxiation*

Ratio of the A.P. in nitrogen to the A.P. in oxygen is given in column 5. The D.P. is listed in (column 6).

NERVE	TIME IN MIN.	PERCENTAGE REDUCTION OF A.P.		$\frac{\text{A.P. IN N}_2}{\text{A.P. IN O}_2}$	D.P. IN mV
		O <sub>2</sub> side	N <sub>2</sub> side		
Cat peroneal	0	0	0	1.000	0
	7	18	33	0.820	1.0
	10	50	73	0.540	1.6
	13	75	83	0.680	2.6
	15	89	93	0.640	3.4
	20	100	100	0.000	5.6
Rabbit peroneal	0	0	0	1.000	0
	4	0	0	1.000	0.4
	10	18	31	0.840	3.6
	15	91	94	0.670	8.0
	20	100	100	0.000	10.4
Rabbit tibial	0	0	0	1.000	0
	5	19	9	1.120	0
	10	48	44	1.075	1.5
	15	67	70	0.910	3.5
	20	86	91	0.640	5.0
	25	95	96	0.800	7.0
	30	100	100	0.000	7.5
Cat saphenous	0	0	0	1.000	0
	5	-9	-3	0.950	0.2
	12	-12	-7	0.950	0.4
	19	8	45	0.600	0.8
	23	15	52	0.565	1.4
	26	18	58	0.510	2.0
	30	37	77	0.365	2.8
	35	73	94	0.162	3.6
	37	88	97	0.250	3.8
	38	94	100	0.000	3.6
Bull-frog	0			1.000	
	40			1.000	
	75			0.834	
	100			0.680	
	130			0.640	
	150			0.550	
	165			0.562	
	180			0.635	
Bull-frog	0			0.800	
	60			0.783	
	90			0.705	
	120			0.700	
	150			0.685	
	180			0.705	
	210			0.750	

then subjected to nitrogen (9) and the spike of one fiber recorded by stimulating at the threshold value of the most sensitive fiber. No partition was used in these tests. The results of one experiment are illustrated in figure 7. The amplitude of the action potential decreases from about 10 mV to 5 mV in 72 minutes, proving that the "all" of the individual nerve fiber is reduced by asphyxiation. Also there is a very definite decrease in the conduction velocity from 2.5 M/sec. to 1.2 M/sec. The results from other crustacean preparations showed that the A.P. and velocity decrease 20 to 50 per cent in nitrogen before blocking occurs.

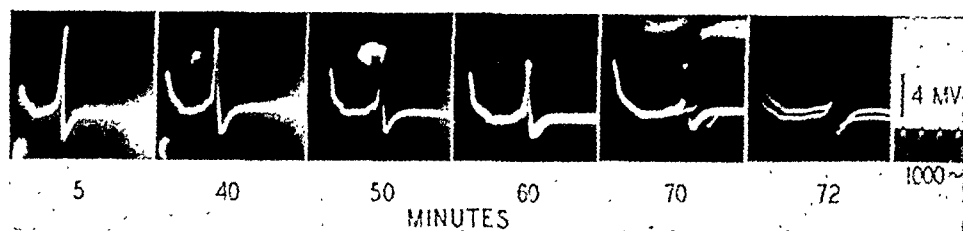


Fig. 7. Action potential from single fiber of lobster nerve. The fiber was placed in a nitrogen atmosphere and recordings made after 5, 40, 50, 60, 70, and 72 minutes. The amplitude and conduction velocity decrease about 50 per cent in 72 minutes when blocking occurs.

**DISCUSSION.** *Nitrogen.* The oxygen supply is rapidly depleted from an area of nerve in nitrogen, and that nerve portion becomes electro-negative relative to an area in oxygen. This effect has been observed by Gerard (4) in frog nerve. This potential change indicates that the individual fibers in nitrogen are depolarizing and as this takes place the amplitude of the action potential diminishes. The A.P. diminution is brought about in three ways.

A. The spike size of the individual fiber decreases. This observation is in support of the concept that the spike is related to the membrane potential, which diminishes during anoxia.

B. In the absence of oxygen, the depolarization continues eventually reaching a critical value beyond which propagation of an impulse ceases. This is when blocking occurs even though the A.P. of some single fibers may still be 75 per cent original size and the depolarization only 50 per cent of the maximum. After blocking takes place the depolarization may continue for quite some time before reaching the maximum value.

C. The decrease in the velocity of conduction during anoxia diminishes the overall action potential of the nerve by spreading out the A.P. of single fibers and thereby eliminating much of the summation of spikes originally traveling at the same speed.

These three effects are all rapidly reversible when the oxygen supply is replenished. The single fiber spike increases, the blocked fibers start to conduct, and the conduction velocity increases to the common speed for that class of fiber bringing about summation.

The threshold of excitability remains constant during mammalian nerve asphyxiation.

*Carbon monoxide and HCN.* Both CO and HCN are oxidation inhibitors.

Schmitt has observed (6) that a 30 per cent concentration of CO in oxygen extinguishes nerve activity in about the same time and manner as nitrogen. This suggests that the inhibitor action of the gas simply asphyxiates the nerve by preventing the use of the oxygen in the surrounding medium. It would be expected that 100 per cent CO would eventually extinguish the A.P. by simple asphyxiation. In the present work the majority of effects observed using 100 per cent CO or 10 per cent HCN in air were identical to those recorded in nitrogen. In some mammalian nerves, activity was extinguished in a shorter time with 100 per cent CO than has been recorded in nitrogen, possibly indicating that the process of extinguishing the nerve activity by oxygen lack in the pure CO atmosphere is being assisted by the inhibition of oxygen utilization. The 10 per cent HCN mixture leaves at least 18 per cent oxygen in the medium surrounding the nerve, a more than ample amount for normal nerve activity, yet the nerve is prevented from using this oxygen by the enzyme inhibiting action of the cyanide, and extinction times are observed equal to those witnessed in nitrogen asphyxiation.

It is concluded that whether oxygen is simply removed from the nerve tissue by physical replacement, or that its use by the tissue is prevented, the result is the same—arrest of  $O_2$  utilization. This conclusion is supported by the fact that extinction times are all about the same in  $N_2$ , 10 per cent HCN or 100 per cent CO. If an energy reserve of some kind does exist (as postulated by many early workers (6)) for maintaining normal nerve activity in the absence of  $O_2$ , it evidently can be used for this function in the presence of the oxidation inhibitors. The action of the asphyxiants nitrogen, carbon monoxide and cyanide, may be explained assuming that the nerve uses oxygen to manufacture this hypothetical energy reserve. The asphyxiants, then, would prevent this manufacture, causing the reserve eventually to become exhausted.

*Alcohol.* The alcohol vapor used was found to stop all nerve activity in 10 to 20 minutes. Depolarization occurs, the A.P. decreases and the threshold rises slowly, even though oxygen is present in high concentration. In one experiment the spike of a single fiber was observed to decrease 15 to 20 per cent before blocking occurred, confirming Davis et al. (2). It seems likely that alcohol acts directly on the chemical structure of the nerve fiber membrane causing the depolarization and subsequent loss of electrical activity.

*Ether.* The effects recorded when nerves were exposed to dilute ether vapor were the same as those for alcohol. In high concentrations of ether, the A.P. vanished in 3 to 4 minutes, and little or no depolarization is seen. This may indicate that the narcotics can block conduction not only as a result of depolarization, but by a direct action on the conduction mechanism.

The gases and vapors examined can be divided in two groups, those preventing oxygen utilization—nitrogen, CO and HCN—and narcotics—alcohol and ether. Though both groups seem to be able to stop the conduction of action potentials by depolarization of the nerve fibers, there are some differences in the effects produced by these two classes of agents. The most obvious difference is in the respective effects on nerve excitability. The narcotics cause the threshold to

rise slowly and continuously during narcosis, whereas the asphyxiants bring about little change in the threshold until the sudden rise at extinction of conduction. In  $O_2$  after narcosis the threshold recovers only slowly, but after asphyxiation attains the normal value abruptly. An explanation of this remarkable difference is not available at present.

The author wishes to thank Professors A. van Harreveld and C. A. G. Wiersma for their advice and many helpful suggestions throughout the work.

#### SUMMARY

The results of experiments in which mammalian and frog nerves were exposed to nitrogen, cyanide, carbon monoxide, alcohol and ether are presented.

When asphyxiated ( $N_2$ , CO, HCN), the nerve depolarizes with subsequent reduction in size of the action potential. The excitability remains unchanged.

The action potentials of asphyxiated single fibers from lobster nerve actually decrease as much as 50 per cent in size, and the velocity of conduction also is diminished 50 per cent before blocking takes place. Experiments were run indicating that the A.P. of mammalian nerve fibers diminish in nitrogen.

Nitrogen, CN and CO all act as asphyxiants either by removing all oxygen from the tissue, pure nitrogen and pure CO, or by inhibiting oxidation reactions, 10 per cent HCN in air, and CO (6).

When narcotized by dilute mixtures of alcohol or ether in oxygen, the nerve depolarizes, the action potential decreases and the excitability diminishes. Activity extinction is brought about by narcosis in about the same manner as by anoxia. High concentrations of these drugs greatly shorten the extinction time.

The narcotics, alcohol and ether, probably act directly on the chemical structure of the nerve causing the depolarization and extinction of the action potential. This action is carried on even with a large percentage of oxygen present.

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# DIABETES IN PARABIOTIC RATS<sup>1</sup>

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Numerous investigations have yielded results which support the hypothesis that hyperglycemia produced by glucose infusion (1) or that administration of anterior pituitary extracts (APE) stimulates the islet cells of the pancreas to secrete more insulin. Administration of APE in the adult dog causes hyperglycemia and eventual exhaustion of the islet cells (2, 3). In rats or in young puppies APE produces a hyperplasia of islet tissue without apparent increases in blood glucose (4, 5). Most investigators have supported the conclusion that the pancreatic changes were in direct response to the hyperglycemia from glucose infusions or to the blood-sugar raising effects of the APE.

Since Dunn, Sheehan and McLetchie (6) and numerous other investigators (7, 8, 9) have shown that alloxan destroys the islet cells of the pancreas, it became of interest to determine whether prolonged hyperglycemia produced by administration of a diabetogenic dose of alloxan in one partner of parabiotic rats would cause the production of sufficient insulin by the pancreas of the non-diabetic parabiont to alleviate the hyperglycemia of the diabetic one.

**MATERIALS AND METHODS.** Male-male or female-female pairs of littermate rats, obtained from Sprague-Dawley, Inc., were united in parabiosis at 31 days of age, using the technique of Bunster and Meyer (10) except that metal skin clips were used instead of sutures in closing the skin incisions. Diabetes was produced in one member of a pair by the administration of 100 mgm./kgm. doses of 5 per cent alloxan injected into a tail vein on the second day after the surgical union, as anastomosis of blood vessels does not occur in parabiotic rats until the third day of union (11). Under these conditions there would not be any transfer of alloxan on the second post-operative day.

The blood sugar determinations were made before and at various times following the injections, using blood obtained from the cut tip of the tail, and the method of Jegher and Myer (12) adapted to the Evelyn photoelectric colorimeter. The rise and fall of blood glucose in both the parabionts were followed, at first daily, then later on alternate days, for periods of three or more weeks if the animals survived.

**RESULTS AND DISCUSSION.** The first part of this study includes observations made on twenty-seven pairs of male-male rats, with one partner alloxan-treated, but not receiving any exogenous insulin. A summary of all the blood sugar values found during the period will be found in table 1. Twelve of the pairs died

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before the end of the three week period of blood glucose analysis. Six of the twelve injected partners developed marked hyperglycemias, which were greatly reduced before the end of their survival period, which varied from thirteen to seventeen days after the alloxan injection. Four other parabiotic pairs, living for five to sixteen days after alloxan treatment, showed a great hyperglycemia during the entire time, while the injected rats of two pairs had only mild diabetes. Figure 1(a) serves to illustrate the cases discussed, in which no reduction of blood glucose occurred, and figure 1(b) and 1(c) illustrate those in which marked reduction followed anastomosis of blood vessels. This observation was in marked contrast to the data obtained for single diabetic rats (unpublished data) and for diabetic-diabetic pairs of rats to be discussed later in this paper. In neither of

TABLE 1

*Blood glucose in male parabiotic rats, after the right partner had been made diabetic by alloxan*

PARTNER	DAYS AFTER THE INJECTION OF ALLOXAN											
	1	3	5	7	9	11	13	15	17	19	21	23
Left	27*	27	26	24	26	26	26	22	20	16	16	15
	194†	181	190	176	166	164	162	159	136	169	168	132
	(119-	(131-	(120-	(106-	(119-	(125-	(124-	(95-	(76-	(131-	(120-	(101-
	293)‡	266)	395)	379)	238)	241)	288)	231)	259)	213)	306)	171)
Right	27*	27	26	24	26	26	26	22	20	16	16	15
	188	391	455	340	329	285	301	334	325	315	326	284
	(143-	(144-	(124-	(138-	(44-	(157-	(128-	(155-	(123-	(143-	(151-	(76-
	265)	731)	823)	600)	489)	436)	433)	710)	601)	444)	418)	420)

\* Number of animals.

† Average blood glucose, mgm. per cent.

‡ Range blood glucose, mgm. per cent.

these groups was there reduction of blood glucose values during the period of observation.

Of the fifteen pairs of parabiotic rats which survived through the three weeks period of testing, or longer, eight rats showed marked hyperglycemia after the alloxan injection, but the hyperglycemia decreased during the period of observation. In the other seven pairs, the injected rat exhibited moderate diabetes, which fluctuated in degree, but did not become consistently reduced during the three weeks period. Figure 2(a) and (b) show the course of the blood glucose changes in two of the parabiotic pairs, in which a marked reduction from the original high values can be traced. Figure 2(c) is representative of the group in which the diabetic animal was not obviously protected by the non-diabetic one.

The evidence shows that reduction of hyperglycemia occurred in fourteen out of twenty-seven diabetic rats in parabiosis with normal rats, and a small reduction in hyperglycemia in four other pairs. The other rats in the series either died with severe hyperglycemia, or exhibited only mild hyperglycemia which was not significantly reduced during the course of observation.

With these data available it became of interest to know whether exogenous insulin could cross from one parabiont to the other, and if so, whether the circulatory connections were adequate to transport insulin at a rate rapid enough to cause any marked decrease in the hyperglycemia of the diabetic partner. To answer this question exogenous insulin was administered to the non-diabetic partner of four male-male parabiotic rats and glucose determinations were made on both partners before, and four to six hours after the injections had been made. The non-diabetic partner was injected subcutaneously with four units of protamine zinc insulin and blood glucose was determined four hours after the injection of insulin. Notable decreases in blood glucose were found in both the non-diabetic injected and diabetic partners. The glucose values before and after insulin administration and the net changes are summarized in table 2. The average change in blood sugar in the injected non-diabetic rats of four pairs can be seen to be  $-62$  mgm./100 cc., while the change of  $-130$  mgm./100 cc. in the diabetic

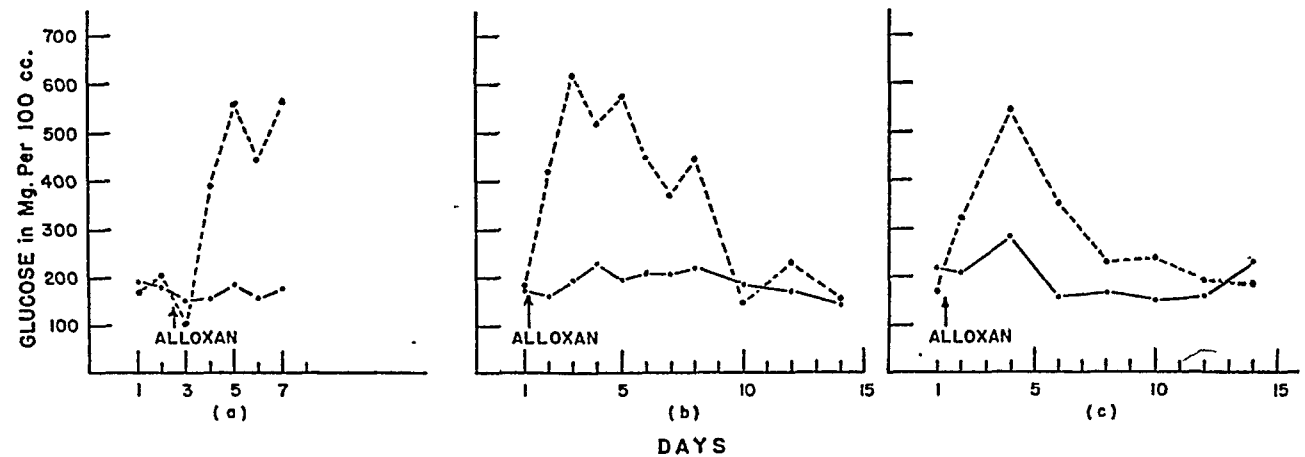


Fig. 1. Blood glucose values for three pairs of male-male parabiotic rats, one partner diabetic ----, the other partner non-diabetic ——. (a) decrease in hyperglycemia in the diabetic partner. (b) and (c) Marked decrease in hyperglycemia of diabetic partner.

partner was almost twice as great. Using the same four pairs of rats a few days later, injections of 12 units were made into the non-diabetic partners, after blood samples had been taken for glucose determinations; six hours later blood samples were taken again (table 2). The results show the same tendency toward reduction of hyperglycemia, but the absolute changes were less in the diabetic rat than when 4 units of insulin were used. There seems to be no adequate explanation for these differences. The data indicate, however, that insulin is transferred from one parabiont to the other and that adequate blood vessel anastomosis had been established for its transfer from non-diabetic to diabetic rats.

In view of the decrease in the degree of the diabetes in some of the alloxan injected parabiotic rats, with little change in degree in other diabetic partners, it became of importance to determine the effects of separation of the parabiotic rats upon the blood glucose curves of the partners and to ascertain whether the normal parabiont had actually been protecting the diabetic member, or if the severity of diabetes had spontaneously subsided. In addition, such a surgical separation

would provide a means for determining whether the islet tissue of the normal partner had hypertrophied to the extent that hypoglycemia in the normal rat would follow the separation of the parabionts. Therefore, separation was per-

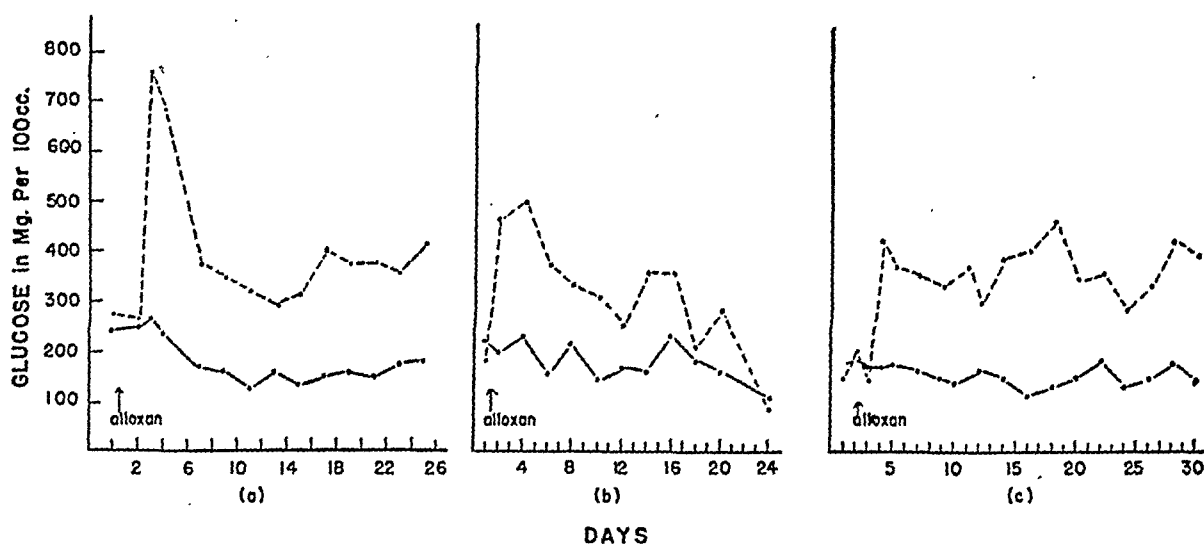


Fig. 2. Blood glucose values for three pairs of male-male parabiotic rats, one partner diabetic ----, the other non-diabetic ———. (a) and (b) Marked decrease in hyperglycemia of the diabetic partner. (c) No reduction in hyperglycemia of the diabetic partner.

TABLE 2

*Blood glucose after insulin administration to the diabetic partner of male parabiotic rats*

	INSULIN-TREATED NON-DIABETIC PARTNER			UNTREATED DIABETIC PARTNER		
	Blood glucose: mgm. %			Blood glucose: mgm. %		
	Before insulin	After insulin	Change	Change	After insulin	Before insulin
4 u. protamine zinc insulin, sub.; blood glucose 4 hrs. later	155	80	-75	-179	216	395
	138	69	-69	-100	253	353
	150	96	-54	-145	251	396
	151	102	-49	-94	281	375
Average (4).....	149	87	-62	-130	250	380
12 u. protamine zinc insulin, sub.; blood glucose 6 hrs. later	189	101	-88	-34	401	435
	165	91	-74	-62	439	501
	188	115	-73	+24	449	425
	175	101	-74	-33	338	371
Average (4).....	179	102	-77	-26	407	433

formed by operation upon four pairs of parabiotic rats. Rats in the separated group included two pairs which had shown evidences of decreased diabetic symptoms, and two others in which alleviation of diabetes was slight or variable.

Figure 3 (a, b, c) is of the blood glucose values for the male-male pairs of separated rats, and covers the time from parabiosis through the period following

separation. Examination of this figure discloses that increases in hyperglycemia in diabetic rats followed the surgical separation of the non-diabetic partners. By observation it was seen that gross symptoms of diabetes also became more extreme. For example, the inequality in body size became even greater in these separated pairs; the diabetic rats became more emaciated, with loss of weight, loss of hair, and enlargement of viscera to the extent that an actual splanchnomegaly existed. Emaciation, sparseness of body fur, and splanchnomegaly were also typical of single rats with long-standing alloxan diabetes, and was in general, proportional to the severity of the diabetic symptoms.

Changes in the blood glucose of the non-diabetic parabiotic rats were not great and, except for a few animals which showed slightly elevated blood glucose early

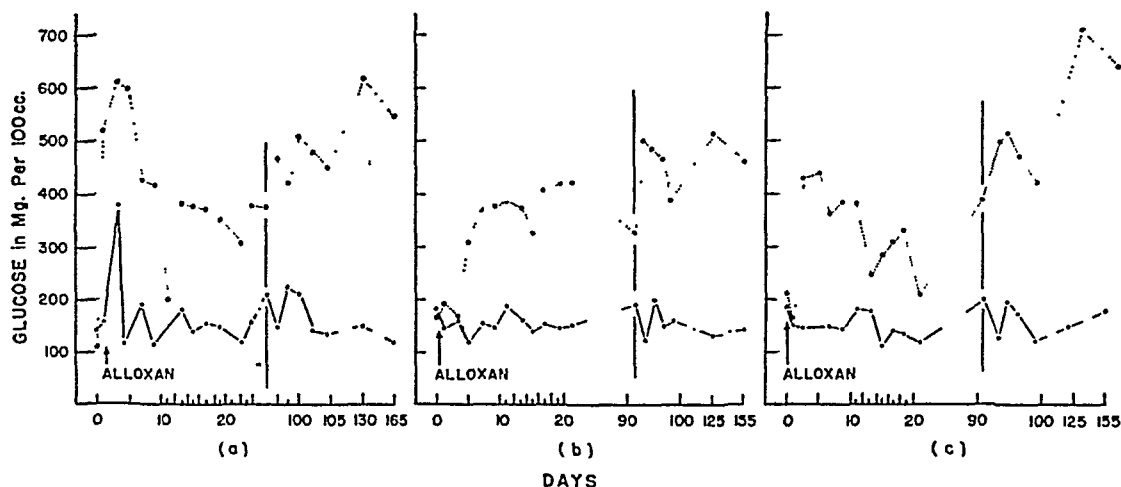


Fig. 3. Blood glucose values for separated partners of three pairs of male-male parabiotic rats. Diabetic rat ----, non-diabetic rat —; vertical line indicates day of Separation.

in parabiosis at the time the diabetic partner was extremely hyperglycemic, there were no changes that could be considered significant. Separation of parabiotic rats did not produce any evidence of hypoglycemic reactions in the non-diabetic rats.

The evidence for alleviation of diabetes in some of the diabetic—non-diabetic parabiotic rats and the further increase in severity of diabetes after separation are in accord with the results reported by Forschbach (13a, b) for parabiotic dogs in which one member was diabetic. Forschbach's dogs, however, had coelio-anastomosis, with greater opportunity for glucose to diffuse across in the body fluids, in addition to crossing by vascular channels. Thus there was probably greater stimulation of the pancreas of the normal dog from the increased blood sugar than could be expected in the parabiotic rats with limited vascular anastomosis. It appears likely that the reduction in diabetes in these parabiotic rats was limited by the extent of infusion of glucose from the diabetic to the non-diabetic partner, and/or the concomitant passage of insulin from the normal back to the diabetic member of the pair.

Evidence for transfer of insulin from the non-diabetic rat to the diabetic member is to be found in: 1, reduced hyperglycemia in the diabetic member of parabiotic rats; 2, the increased hyperglycemia when diabetic rats were separated from non-diabetic partners, and 3, the decrease in the hyperglycemia of a diabetic rat when insulin was injected into the non-diabetic partner.

To obtain additional evidence that the reduction in hyperglycemia can be produced by the transfer of insulin from the normal to the diabetic partner by way of the circulatory connections between the pairs, another experiment was done in which thirty-one day old littermate female rats were joined in parabiosis, and on the second post-operative day, both members of the pairs were given 0.2 cc. of 5 per cent alloxan by tail vein injection. The eight pairs of rats used for this experiment had significantly higher blood sugar values throughout the experiment than pairs in which only one partner was diabetic, and did not show the tendency toward reduction of hyperglycemia that had been noted in the pairs with one

TABLE 3

*Blood glucose in female parabiotic rats in which both partners had been made diabetic by alloxan*

NO. OF RATS	TREATMENT	BLOOD GLUCOSE: MCM. %		
		Highest values av.	Lowest values av.	Overall mean
16 R.P.	Alloxan	463	218	329
16 L.P.	None	160	139	148
8 R.P.	Alloxan	1071	677	877
8 L.P.	Alloxan	993	793	896
16 R.P.	None	160	111	139
16 L.P.	None	149	117	133

partner diabetic and the other possessing a normal pancreas. Table 3 is a summary of the high, low, and mean blood glucose determinations in the experiments with female parabiotic rats. The data show the greater severity of diabetes when both partners were treated with alloxan.

It was desirable at this point to test further the transfer of exogenous insulin from an injected parabiont to its uninjected partner, using pairs of rats in which both partners were diabetic, so as to rule out the complications of endogenous insulin. Therefore, one member of ten diabetic-diabetic pairs of rats were given doses of insulin varying from 1 to 3 units, administered subcutaneously or intravenously. Blood samples for glucose determinations were taken at times ranging from twenty minutes to four hours after the injection. The results are shown in table 4. From the data it can be seen that a dose of 1 unit of insulin was ineffective at two to three hour intervals after injection. Both 2 and 3 units of insulin were effective; the most marked effect was obtained when 3 units of insulin were injected subcutaneously and blood samples were taken after a four hour interval

when the blood glucose was reduced 616 and 543 mgm./100 cc. in the left and right partners respectively. These results constitute further evidence that insulin can be transferred in parabiotic rats, and that it can effectively reduce hyperglycemia in the non-injected partner.

In summation, it has been shown that high blood glucose levels were not maintained in those diabetic rats untreated with insulin with a normal partner, and they afford additional evidence that the diabetic rat is partially protected by the pancreas of the normal partner. Diabetic-diabetic pairs of rats did maintain higher blood sugar levels than diabetic-normal pairs, which further supports the conclusion just made. Exogenous insulin given to one diabetic rat reduced the glucose levels in the blood of both members, and added to the evidence that insulin is transferred.

TABLE 4

*Blood glucose in female diabetic parabiotic rats after injection of insulin into one partner*

NO. OF PRS.	INSULIN TREATED DIABETIC RP				UNTREATED DIABETIC LP		
	Treatment	Blood glucose			Blood glucose		
		Before insulin	After insulin	Change	Change	After insulin	Before insulin
3	1 u. insulin s.c. bl. glu. 2-3 hrs.	1074	918	-156	+229	1270	1041
2	2 u. insulin s.c. bl. glu. 1 hr.	533	117	-416	-267	158	425
2	2 u. insulin i.v. bl. glu. 20 min.	1373	1255	-108	-30	1258	1288
3	3 u. insulin, s.c. bl. glu. 4 hrs.	795	179	-616	-543	363	906

Organ weights of diabetic rats showed testes, ovaries and uteri to be below normal size, the thymus was atrophied and the kidneys were hypertrophied (data to be published elsewhere).

The parabiotic rats which survived without insulin treatment were allowed to remain in the laboratory and in the course of a number of weeks it became apparent that disproportionate rates of growth existed between the members of the pair. The growth of the diabetic partners varied somewhat according to the severity of the diabetes, the slowest rate of growth occurred in a rat in which hyperglycemia was very great at the beginning of diabetes, but decreased later.

At irregular times ranging from approximately six to twelve weeks after the injection of the alloxan, complete bilateral cataracts appeared in the diabetic partners of all the surviving pairs, but no cataracts developed in the non-diabetic members. The onset of the cataractous condition appeared to be rather sudden. Occasionally an opaqueness was apparent before the cataract became complete,

but this lasted for only one or two days and was followed by the cataracts developing over the entire lens, so that the eye appeared white, with tracings of blood vessels over its surface.

#### SUMMARY

1. Parabiotic rats with one partner made diabetic by alloxan were used to study the effect of constant hyperglycemia on pancreatic hypersecretion. Approximately 50 per cent of the diabetic rats showed markedly reduced hyperglycemia during the test period. Exogenous insulin given to the non-diabetic partner reduced the hyperglycemia in the diabetic partner, indicating that circulatory connections were sufficient for insulin transport.

2. Surgical separation of the parabiotic rats produced an increase in the hyperglycemia of the diabetic rats, and exaggerated the other diabetic symptoms. Hypoglycemia, however, was not observed in the normal partner after separation.

3. Parabiotic rats with both members diabetic were continually in severe diabetes, and no alleviation occurred except when exogenous insulin was given.

4. Exogenous insulin given to one partner of diabetic-diabetic parabiotic rats reduced the blood glucose in both members of the pair.

5. Complete bilateral cataracts and subnormal development of the gonads were observed in diabetic members of parabiotic rats which survived for a number of weeks; the cataracts appeared at approximately 8 to 12 weeks after the injection of alloxan.

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# THE INFLUENCE OF AGE ON THE HEMOCONCENTRATING RESPONSES OF THE DOG

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The development of homothermism in newborn mammals is accompanied by a number of physiological adjustments, such as shivering, metabolic responses, and other protective mechanisms against heat and cold that appear during the first few days or weeks of life. A well-authenticated response to cold in adult homothermal animals is the shifting of fluid from the blood to the body tissues, thereby increasing the concentration of the blood. Barbour, McKay and Griffith (1943) demonstrated that such cold anhydremia occurs within a period of minutes if the body temperature is lowered somewhat, either with or without anesthesia. When adult dogs were made poikilothermal by sectioning of the spinal cord, however, Barbour and Tolstoi (1923) found that hemoconcentration did not occur in response to cooling.

A survey of the literature disclosed that while the blood concentrating mechanism is undoubtedly characteristic of adult animals, as shown by Barbour and associates, an investigation of this response during the first few days or weeks of postnatal life in the dog apparently has not been undertaken. For this reason, and because of the increasing interest in cold therapy and refrigeration anesthesia in human patients, the experiments below were carried out.

**METHODS.** 1. *Cooling with ether anesthesia.* All animals used in the investigation were mongrel dogs maintained in good health as far as could be ascertained on a diet of commercial dog biscuits. The immature dogs we used were, for the most part, born and raised in the University of Missouri Medical School animal house. Each dog was used only once in the investigation, except in some instance those cooled to 30°C. were permitted to recover, and used again in an older age group when not less than 7 days elapsed between the two blood samplings.

Blood samples were drawn from the femoral vein or by means of cardiac puncture. The specific gravity of whole blood and serum was determined by the falling drop method of Barbour and Hamilton (1926). Hemoglobin readings were made with the aid of a Cenco-Sheard-Sanford photometer, and red blood cell counts made with a Neubauer counting chamber. The hematocrit readings were made with the Van Allen tube, since the small animals used made it advisable to keep blood withdrawals at a minimum in order to avoid complications arising from low blood volume.

The procedure of the experiments was as follows: A blood sample was taken for the normal readings at a room temperature of about 25°C. The animal was then deeply etherized and immersed to the level of the neck in a bath of about 5°C.



Additional ether was given as needed to maintain anesthesia during the cooling process until the deep rectal temperature had fallen to the anesthetic level of about 25°C. Cooling was then continued without ether until the onset of respiratory failure indicated that death had occurred. The temperature level at which respiration stopped varied with the age of the animal. Those under 20 days showed respiratory failure at an average of 10°C., and those between 21 days and 6 months at an average of 15°C. Usually the heart continued to beat long after respiration had stopped.

The time required for the animals to reach the lethal temperature varied with the body volume, the larger dogs obviously requiring more prolonged cooling. In experiments involving small dogs, the rate of cooling was retarded either by using a bath somewhat above 5°C. at the start or by removing the animals from the bath at intervals to delay the fall in temperature. In every instance the total cooling process exceeded one hour so that ample time for blood changes to occur was permitted, especially in view of the report by Barbour and Hamilton (1925) that blood concentration due to cold could be detected in the dog within a matter of minutes by the falling drop specific gravity method. The point at which blood samples were taken was decided by the body temperature, the first sample being taken at normal temperature, the second between 30° and 26°, the third between 25° and 21°, the fourth between 15° and 11°, and the fifth between 10° and 6°C., if respiratory failure had not occurred previously.

Control animals in each group were sampled in the same way as the experimental animals, except that after the first sample had been taken and ether anesthesia had been given, the animal was kept warm by application of heat from a lamp to prevent any significant fall in body temperature. Successive blood samples were then taken over a period of an hour or more during which time the dog was kept under ether, but not permitted to undergo body temperature changes of more than about 1°C.

In order to simplify the presentation of the results in graphic form, the blood changes during cooling were computed on the basis of per cent change from the normal readings. The per cent changes in blood and serum specific gravity were then added and averaged to give a single figure for purposes of graphing the results. The averaging of these two readings was considered justified in that Barbour, McKay and Griffith (1943) determined that the blood changes in response to cooling were due primarily to fluid shifts between blood and tissues and, therefore, would cause changes in both whole blood and serum specific gravity. The per cent changes from normal in the red blood cell count, hemoglobin and hematocrit readings were found and likewise averaged to give a single figure, since each is a measure of the relative red cell content of the blood. Data from control animals also were averaged as described above and plotted in the same graph as the experimental data.

**RESULTS.** 1. *Two to six-day-old dogs.* The results from 8 experimental animals in this age group are shown in figures 1 and 2. As the graphs indicate, dogs in this age group failed to concentrate the blood at any level of body temperature at which samples were taken. Dilution of the blood appeared with the

fall in body temperature and became more pronounced as the cooling proceeded toward the lethal level. Ether administration without cooling to 5 control dogs in the same age group likewise failed to cause hemoconcentration.

*Seven to twenty-day-old dogs.* A total of 12 dogs in this age group were subjected to the experimental procedures described and the data resulting plotted in graphs in figures 3 and 4. Dogs of these ages gave definite evidence of hemoconcentration until the rectal temperature approached 20°C., then began to show a hemodilution which became progressively greater as the body temperature

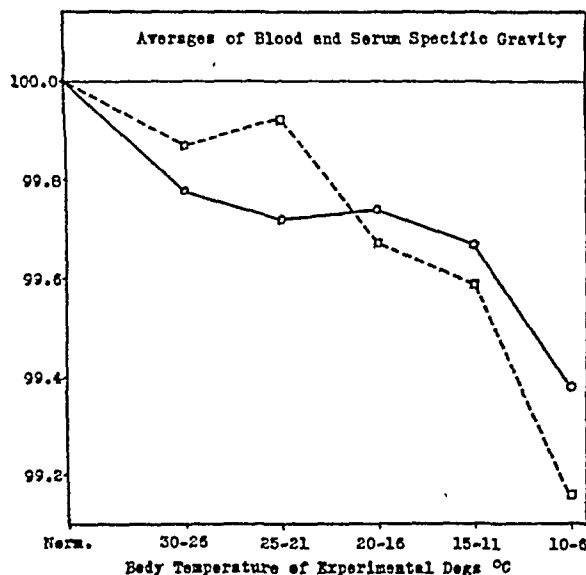


Fig. 1

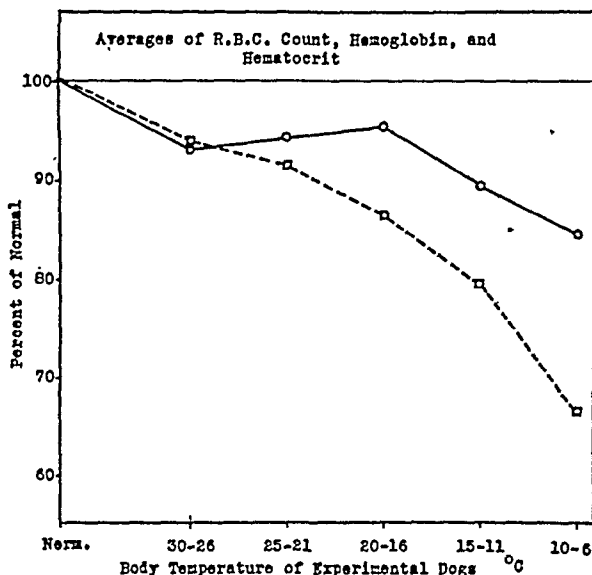


Fig. 2

Fig. 1. Solid line indicates blood and serum specific gravity changes from normal in 8 dogs, 2-6 days old, cooled under ether anesthesia and sampled at various body temperatures. Broken line indicates the same readings from 5 control dogs in the same age group given ether anesthesia but kept at normal body temperature during period of samplings.

Fig. 2. Solid line shows average of hemoglobin, hematocrit, and R.B.C. count in the same cooled animals as in figure 1. Broken line shows the same determinations made in the control animals.

descended toward the lethal point. Six control dogs in the same age group concentrated the blood in response to ether administration but not to as great a degree as the animals subjected to both ether and cooling.

*Twenty-three-day-old to one-hundred-fifty-seven-day-old dogs.* The 21 animals in this group responded to cooling by hemoconcentration much as did the next younger group described above. The graphs in figures 5 and 6 indicate that the peak of concentration was reached in the same temperature range as for the 7 to 20-day-old dogs but, for unknown reasons, this older group of animals did not concentrate the blood to quite as great a degree as did the younger dogs. As the cooling became more profound, hemodilution appeared and continued until the lethal temperature was reached.

The administration of ether to 10 control dogs in this group resulted in hemoconcentration. However, this was somewhat less than that occurring in dogs subjected to both ether and cooling.

**METHODS. 2. Cooling without anesthesia.** The first section of this report described the effects of lowering body temperature on blood concentration under

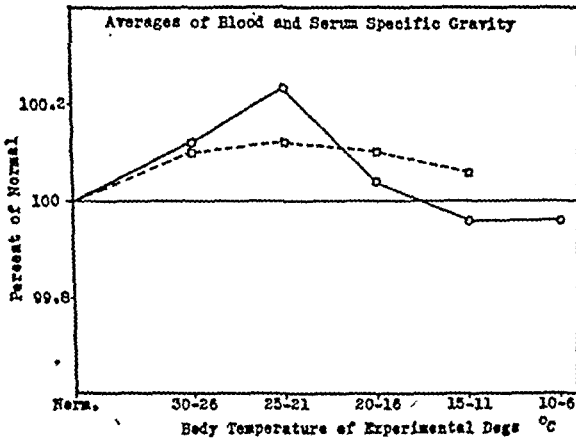


Fig. 3

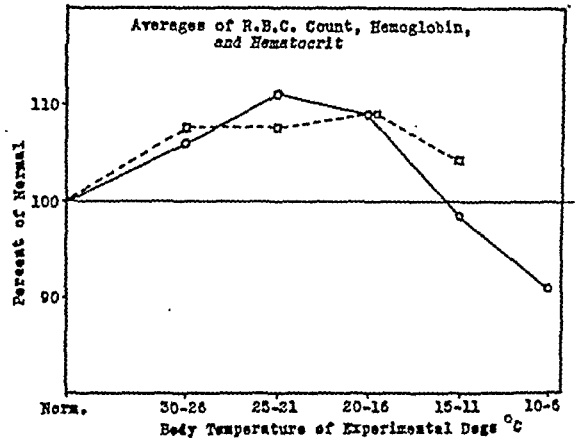


Fig. 4

Fig. 3. Solid line shows changes from the normal in blood and serum specific gravity of 12 dogs, age 7-20 days, given ether anesthesia, cooled, and sampled at various body temperatures. Broken line indicates specific gravity readings taken from a group of 6 control dogs in the same age category, given ether, but not cooled.

Fig. 4. Solid line depicts changes from the normal in the hemoglobin, hematocrit, and R.B.C. count of the cooled dogs shown in figure 3. Broken line gives the same readings in the control group.

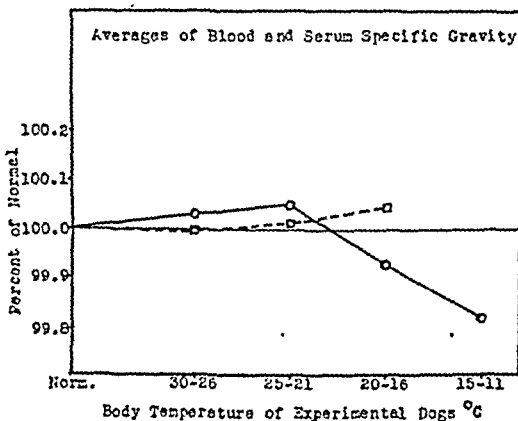


Fig. 5

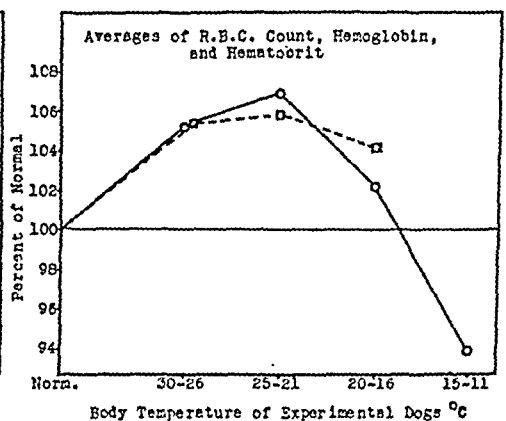


Fig. 6

Fig. 5. Solid line indicates changes from the normal in blood and serum specific gravity of 21 dogs, ranging from 23 to 157 days of age, given ether, cooled, and sampled at various temperatures. Broken line shows the determinations taken from 10 control dogs in the same age group, which were given ether, but not cooled.

Fig. 6. Solid line shows changes from the normal in hemoglobin, hematocrit, and R.B.C. count in the same cooled dogs as in figure 5. Broken line shows the results from the same readings in the control group of dogs.

ether anesthesia. However, ether and other anesthetics have been demonstrated to have a depressing effect on the temperature regulating mechanisms by Britton (1922), Barbour (1923), Woodruff (1941) and others. Moreover, ether administration has been found to produce hemoconcentration in dogs regardless of body

temperature changes by Barbour and Bourne (1923). This hemoconcentrating effect of ether was evident in the control animals recorded in the first part of this paper. Extreme cooling itself also has anesthetic actions, and a pronounced fall in body temperature was found by Barbour, McKay and Griffith (1943) to depress the normal reflex responses to cold, with the result that animals cooled below 23° C. lost the ability to concentrate the blood and showed hemodilution instead. Similar results were found in dogs cooled below 20°C. and in newborn dogs at all temperatures as described in the first part of this paper.

Because of the complicating factors introduced by ether and extreme cooling, it appeared essential to conduct experiments in cooling young animals without ether and without lowering the body temperature to anesthetic levels. Animals of various ages were, therefore, subjected to mild depression of body temperature without the use of anesthesia and the blood changes studied as before.

In the previous experiments it was noted that dogs under the age of 6 to 8 days tended to be more or less poikilothermal, and usually showed a fall in body temperature when exposed to normal room temperatures of about 20 to 25°C. For this reason newborn animals could be cooled several degrees by merely subjecting them to the laboratory room temperature. The dogs older than about 6 to 8 days kept the body temperature fairly constant in the laboratory environment but could be cooled by wetting the skin or by partial immersion of the body in cold water to promote heat loss by vaporization and conduction.

Blood samples were taken for normal readings at the body temperature existing when the dog was removed from the animal house. The animal was then cooled as described above and the second sample taken when the deep rectal temperature was near, but not below, 30°C. Controls of comparable ages were sampled in the same way as the experimentals, except that the body temperature was kept as near the original level as possible by exposure to a lamp or radiator whenever necessary to prevent cooling. The volume removed in blood sampling was kept at a minimum, not more than 0.5 to 0.8 cc. being removed in the total samples taken per animal. By so doing it was anticipated that any hemodilution brought about by blood loss would be negligible, especially in view of the report by Adolph, Gervasi, and Lepore (1933) that a hemorrhage of about 4 cc. whole blood per kgm. of dog failed to produce hemodilution that was detectable by specific gravity and hematocrit methods. Since the smallest dogs used in our experiments weighed about 200 grams or more, the amount of blood removed in the two samplings probably was not a significant factor in determining the results.

**RESULTS.** 2. A total of 56 animals ranging in age from 1 day to 49 days old were subjected to cooling without anesthesia and 18 additional animals in the same age group were used as controls. The data from all animals were calculated on the basis of per cent change from the normal and presented in scatter diagrams in figures 7 and 9 (cooled) and figures 8 and 10 (controls).

From the data presented it is apparent that newborn dogs did not concentrate the blood with any consistency upon cooling but yielded slightly concentrated or diluted blood according to experimental error much as did the controls. With

increasing age, however, the animals responded to cooling by concentrating the blood more consistently, and beyond 1 to 2 weeks of age nearly all animals used had a fairly well developed fluid-shift mechanism.

**DISCUSSION.** It is apparent from the experimental evidence presented above that the hemoconcentrating mechanism which effects a shift in fluid from the blood to the tissues in response to cold does not develop in the dog until several days after birth. The precise time when this mechanism becomes functional was not determined but, during the second week of postnatal life, when there are

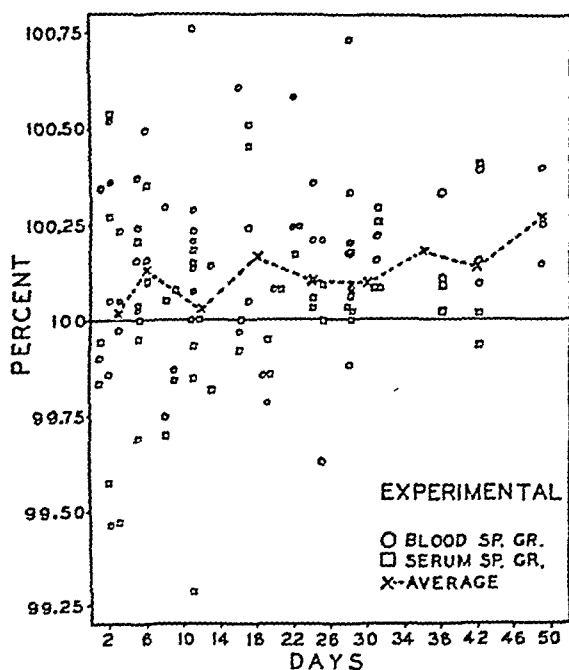


Fig. 7

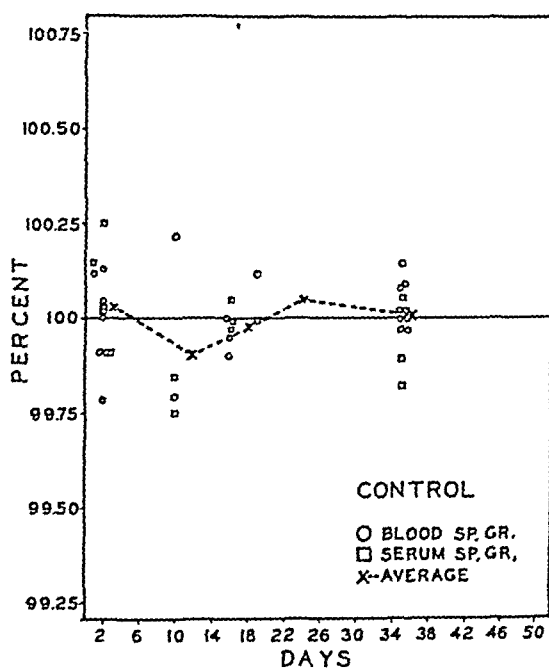


Fig. 8

Fig. 7. Per cent changes from the normal in whole blood and serum specific gravity in dogs cooled to about 30° C. (average 30.5° C.) without anesthesia. Broken line shows averages of blood and serum changes at 1-3 days, 4-6 days, and at 6-day intervals thereafter.

Fig. 8. Per cent change from the normal in blood and serum specific gravity in control dogs kept warm between samples. Broken line indicates averages.

definite indications of the development of homothermism as evidenced by shivering and increased resistance to cold, the ability to concentrate the blood also appears.

The above findings support the suggestion of Barbour and Gilman (1934) that fluid shifts from blood to tissues due to cold are brought about by the increased metabolic demands of the muscle cells used in shivering and in other sites of heat production. Should this be true, it is conceivable that the poikilothermal young dogs that do not show shivering and metabolic responses to cold stimuli should also fail to concentrate the blood as the experimental results have indicated.

In dogs older than 7 days, the responses to cold were similar to those reported by Barbour, McKay, and Griffith (1943) for the adult monkey and the rat in that lowering the body temperature first caused hemoconcentration which was

followed by hemodilution when the body temperature approached anesthetic levels of about 20°C.

The administration of ether also did not produce hemoconcentration in the 2 to 6-day-old dogs but did so in the animals older than this. When cooling was superimposed upon ether anesthesia the degree of hemoconcentration produced was slightly greater than that resulting from either one of these alone but in no group could the effect be doubled by the use of both agents. These observations

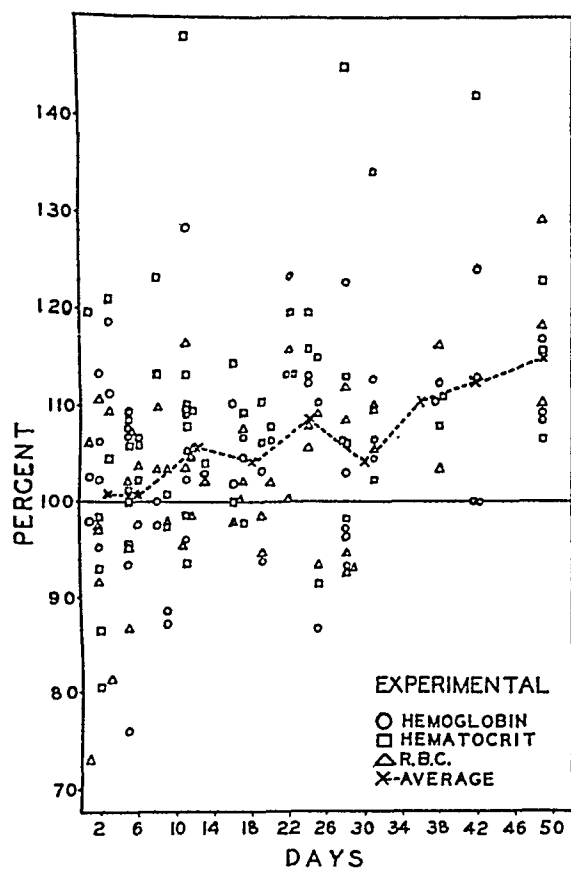


Fig. 9

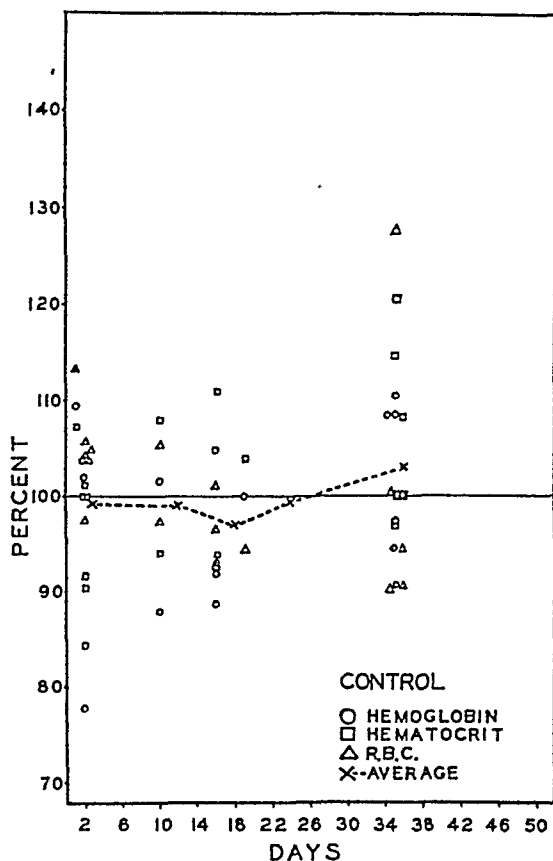


Fig. 10

Fig. 9. Per cent changes from the normal in hemoglobin, hematocrit, and R.B.C. count in same experimental dogs as in figure 7. Broken line shows average of the determinations at intervals of 1-3 days, 4-6 days, and at 6-day intervals thereafter.

Fig. 10. Per cent change from the normal in hemoglobin, hematocrit, and R.B.C. count in the same control animals as in figure 8. Broken line indicates averages.

suggest that the hemoconcentration due to cooling and to ether administration may depend upon similar processes. This is supported by the finding that hemoconcentration caused by cold or ether appeared at about the same time in post-natal development, that each produced somewhat similar degrees of concentration, and that the initial concentration brought about by ether cannot be increased to any considerable extent by lowering the body temperature. Whether the ability to concentrate the blood in response to anoxia or other noxious stimuli appears at the same time in development as the response to ether and cold was not determined. However, the reverse of the above process, the ability to shift

fluid from the tissues to the blood, did appear to be present at birth since dogs in all age groups showed hemodilution when the body temperature was lowered to sufficient levels. It is apparent from the experimental evidence presented that a mechanism for shifting fluid from tissues to blood was present in the newborn dog, but that the reverse of this, the hemoconcentrating mechanism, did not develop until several days after birth and appeared at about the time of the onset of homothermism.

#### CONCLUSIONS

The experimental evidence presented above indicates that the reflex response of hemoconcentration to cold stimulation and ether administration appears at about the time of development of homothermism in dogs. The ability to concentrate the blood in response to these stimuli apparently is acquired during the second week of postnatal life and its appearance is correlated with the development of shivering responses and a general resistance to cooling. Hemodilution, however, was evident at all ages but was found to be greater in young animals than in older ones.

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# THE RÔLE OF THE EXTRACELLULAR FLUID IN TRAUMATIC SHOCK IN DOGS<sup>1</sup>

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When hemorrhagic shock is produced in the dog there is an immediate and continued replacement of the plasma volume (1, 2, and others), whereas in shock following muscle trauma the reduction in the plasma volume remains at a constant level until death occurs (3). This suggests that the fluid which produces the compensatory hemodilution in hemorrhage cannot be utilized in the same way by the traumatized animal. According to Walcott (4) the replacement from body fluids after hemorrhage amounts on the average to 10.7 per cent of the original blood volume. In traumatic shock the situation is somewhat different. The initial increase in volume of the traumatized legs of dogs is followed by a gradual and less marked increase throughout the shock period (5). This change exceeds the total blood volume loss and suggests a continual transfer of fluid to the traumatized areas. The present paper deals with the apparent inability of the traumatized animal to transfer body fluids in sufficient amount to produce any measurable increase in blood volume.

The problem of the mobilization of fluid is concerned with volume changes in the extracellular fluid compartment. We have used two methods to determine deviations from the normal: (a), The changes in serum sodium and chloride, and (b) changes in the fluid available for the solution of sodium thiocyanate. Since our results indicated no significant alteration in the extracellular fluid volume, we next determined what fraction of the water and electrolytes transferred to the traumatized areas represented shifts in extracellular fluid. A comparison was made between the sodium, potassium, chloride and water contents of the entire traumatized hind leg and the corresponding non-traumatized leg. Analyses of skin and muscle from other parts of the body were carried out to determine whether the water loss from these tissues corresponded with the water gain in the traumatized area.

**PROCEDURE.** Observations were made on mongrel dogs of approximately the same weight, 8 to 10 kgm. The animals were deprived of food for 18 to 24 hours before the experiment, but were allowed water *ad libitum*. The thigh muscles were traumatized according to the method of Gregersen and Root (3). The

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clinical course of the resulting shock was identical with that described by these authors. All blood samples were taken from the femoral artery. Determinations were made of the plasma volume with the dye (T-1824) dilution method (6), of the serum proteins using the Abbe refractometer (7) and of the hematocrit value by centrifuging blood in Wintrobe tubes. The water content of whole blood and serum was obtained by drying samples at 78°C to constant weight.

One hind leg of each of 6 dogs was traumatized. At death both hind legs were removed according to the dissection technic of Cullen and Freeman (8). Water content of each leg was determined by the method described by Painter (9). Aliquots of the ground dried tissues were analyzed for sodium, potassium and chloride. Skin samples from the chest and the leg, and muscle samples from the rectus abdominis were taken before and after trauma and again at the time of death. All samples were analyzed for water content and, in some instances, for chloride.

Serum chlorides were determined by the adsorption-indicator method of Saifer and Kornblum (10) and tissue chlorides by the technic of Van Slyke and Sendroy (11). Blood and tissue samples were dry ashed in a muffle furnace and analyzed for sodium according to the method of Butler and Tuthill (12) and for potassium, by the method of Shohl and Bennett (13). The method of Crandall and Anderson (14) as modified by Gregersen and Stewart (6) was used for the determination of the fluid available for the solution of sodium thiocyanate.

**RESULTS.** *Extracellular volume changes.* The effect of muscle trauma on the extracellular fluid volume was estimated from (a) changes in the concentration of the serum sodium; (b) changes in the concentration of the serum chloride; and (c) changes in the fluid available for the solution of sodium thiocyanate. The calculations have been based on serum changes only, any renal loss being neglected since in the traumatized animal the urine flow is so reduced that the chloride excretion never exceeds 2.0 m.eq. (15).

Sodium analyses were done on the serum of 38 traumatized animals. As indicated in table 1, 66 per cent of the animals showed a change from the control of less than 4 m.eq. per liter, 29 per cent showed a change from -4 to -8 m.eq. and 5 per cent, a change of +4 to +6 m.eq. In terms of extracellular fluid this change represents a maximum variation of not more than 5 per cent in any instance, and in two-thirds of the animals the change was less than 3 per cent.

Serum chloride changes were determined in 57 traumatized animals. In 67 per cent of the animals the maximum change from the control was less than 4 m.eq. per liter. In 24 per cent there was a change of +4 to +8 m.eq. and in 9 per cent a change of from -4 to -8 m.eq. (See table 1.) The calculated extracellular fluid changes in the chloride experiments are of the same order of magnitude as those found in the sodium series.

Although the magnitude of the changes in serum chloride concentration is similar to that shown by sodium, the relation of the 2 ions to the severity of shock is different. A possible explanation of this difference can be found in the transfer of chloride from the serum into the red cells during traumatic shock (16). This transfer is associated with the severity of the acidosis, which in turn depends

upon the degree of injury to the animal. Figure 1 shows that an animal which survives 4 hours or less has a slight decrease in serum chloride, whereas an animal living for a longer period of time will have an increase in serum chloride. The serum sodium does not show this relationship.

The time-concentration curves of thiocyanate were determined in the serum of 7 animals before and after trauma. A typical experiment is shown in figure 2. It is apparent that during shock the time required to reach equilibrium is much greater than in the normal animal. If the extracellular fluid volume is calculated before equilibrium is attained, the results show a progressive increase in this value which reaches a constant volume 4 to 5 hours after injection (table 2). In the experiments in which thiocyanate equilibrium has been reached, the values

TABLE 1

*Maximum change from control in serum sodium and chloride following muscle trauma (in m.eq. per liter serum water)*

The changes in electrolytes have been used as the basis for calculating the percentage change in extracellular fluid.

A. Sodium. Total number of dogs—38

No. of dogs.....	6	5	7	6	7	5	2
Change in sodium (m.eq.).....	-8	-6	-4	-2	+2	+4	+6
Per cent change in extracellular fluid.....	+5.6	+4.2	+2.7	+1.4	-1.3	-2.6	-3.9
Per cent of total animals.....	29%		66%				5%

B. Chloride. Total number of dogs—57

No. of dogs.....	2	3	10	10	7	11	10	4
Change in chloride (m.eq.).....	-8	-6	-4	-2	+2	+4	+6	+8
Per cent change in extracellular fluid.....	+7.7	+5.7	+3.7	+1.8	-1.8	-3.5	-5.2	-7.5
Per cent of total animals..	9%		67%				24%	

for extracellular volume are approximately the same as those of the control. In no instance was the maximum change greater than 5 per cent. These findings together with the electrolyte results carried out on the same 7 dogs as well as those mentioned above establish the fact that in traumatic shock no appreciable change in volume of the extracellular compartment occurs.

*Redistribution of extracellular fluid.* The above results show that the inability of a traumatized animal to dilute the plasma compartment cannot be explained by a reduction in extracellular fluid volume. Preliminary determinations of the water content of skin samples taken from non-traumatized areas indicate a decrease in their water content. This suggests that the swelling of the traumatized leg was the result not only of immediate blood loss, but also of the transfer of fluid from the non-traumatized areas. This concept is confirmed by the measurements of the changes in volume of the traumatized extremities (5). Tissue

analysis of water and electrolytes show that except for the water content of the extravasated red cells, the increase in fluid comes entirely from the extracellular fluid compartment.

Table 3 indicates that the increase in water content in the traumatized leg is greater than can be accounted for by the water contributed by the blood which enters the injured tissue. This additional water content varies in most instances from 130 to 150 cc. The extracellular fluid volume of the 2 legs was calculated

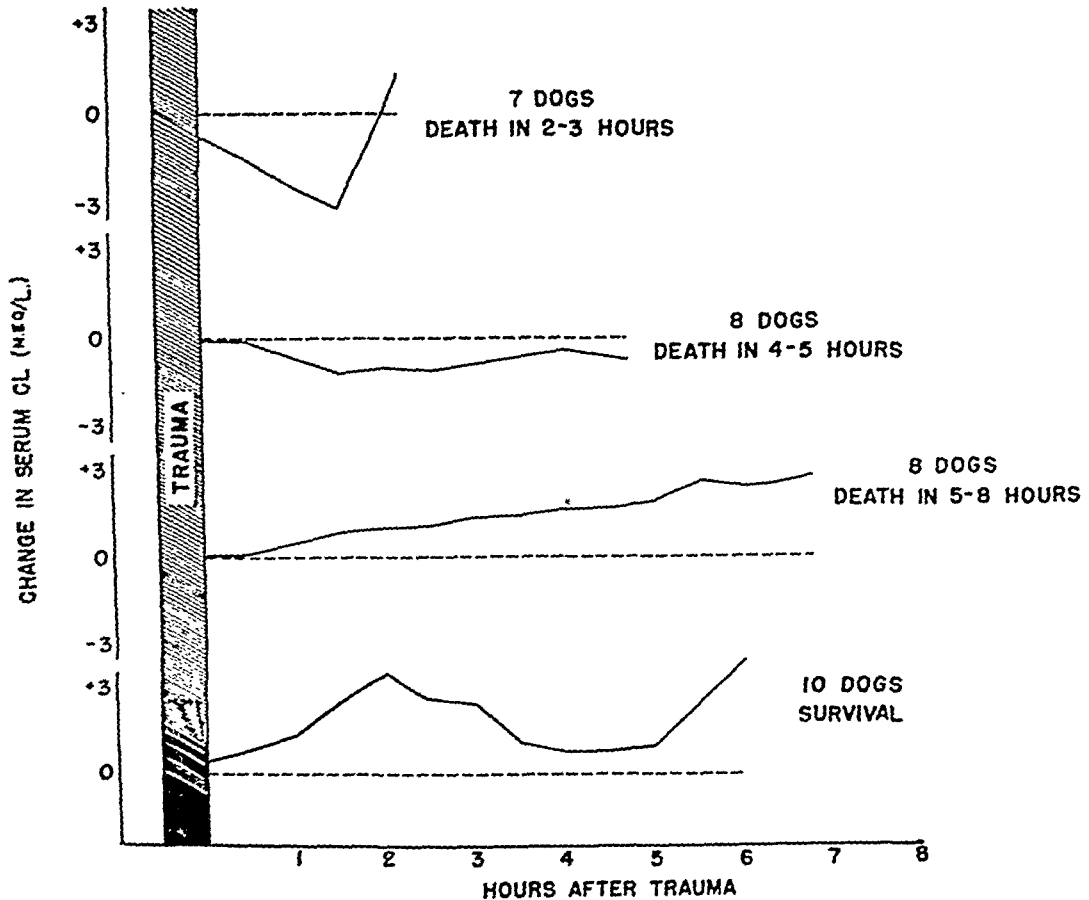


Fig. 1. The relation between the changes in serum chloride concentration and the severity of injury as determined by the length of time which the dog survives muscle trauma. The dotted lines represent the control values; the solid lines, the average change shown by the animals in each group.

from their sodium and chloride content in the manner described by Hastings and Eichelberger (17). From table 4 it can be seen that the difference in calculated extracellular fluid volume between the traumatized and the non-traumatized legs is approximately equal to the difference in water content of the 2 legs. The water content of the non-ruptured extravasated red cells cannot be differentiated from the extracellular fluid, for in dogs it has somewhat similar sodium and chloride concentrations. However, the amount of water contributed by the extravasated cells is so small that it is of very little significance in the final calculation.

Since the size of the extracellular compartment does not change after trauma, thiocyanate measurements made before trauma were used to determine the fraction of the total extracellular volume present in the traumatized and the non-traumatized legs. Table 4, column c shows that according to calculations based on sodium analyses the traumatized leg contains 12.6 to 19.0 per cent, whereas

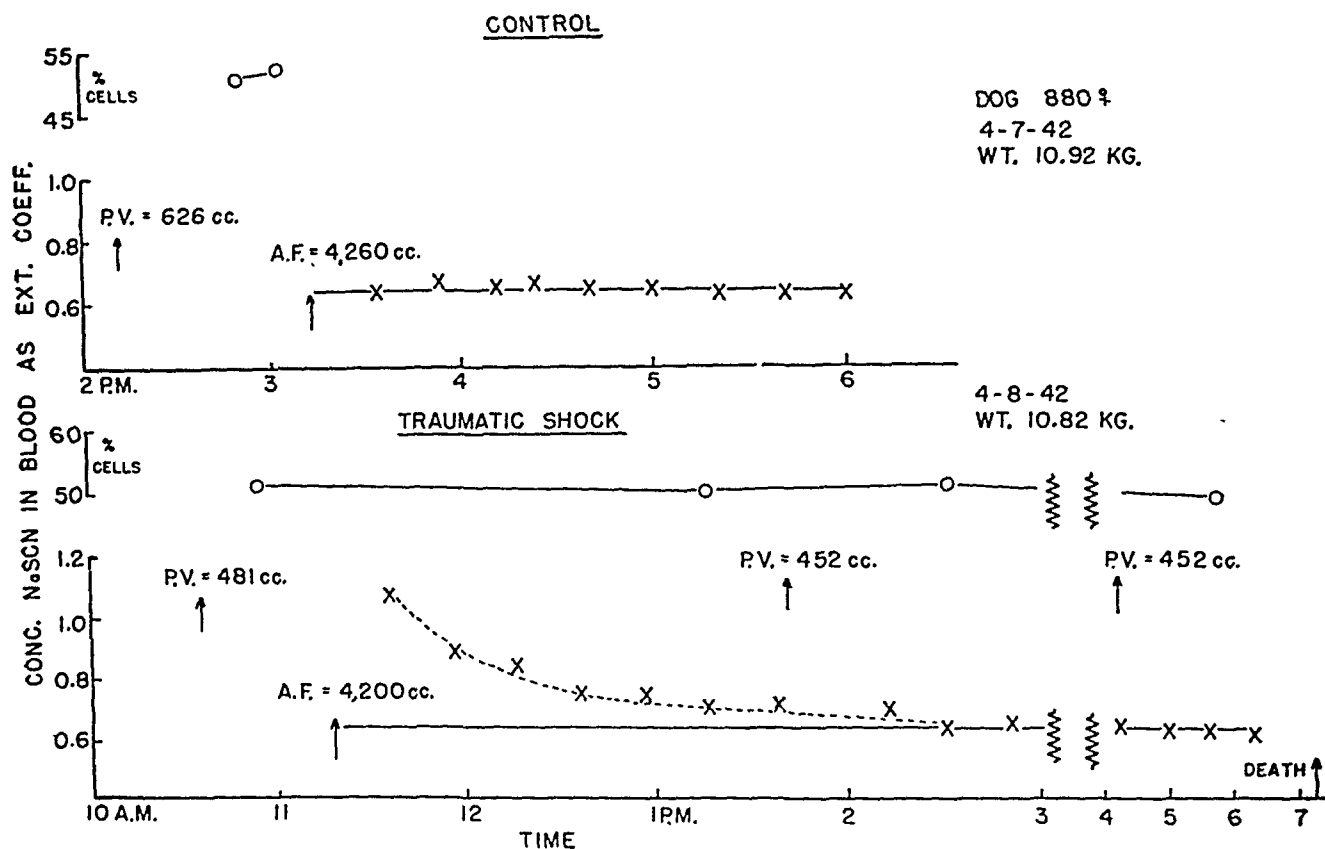


Fig. 2. The time concentration curves (x—x) of the rate of disappearance of thiocyanate from blood serum are shown before and after muscle trauma. The dotted lines indicate the period of "mixing". The solid lines which are extrapolated to the time of injection indicate the concentration values used for the final calculation of available fluid (A.F.). The cell volume index (O—O) as well as the plasma volume (P.V.) values illustrate the constancy of the blood volume following muscle trauma.

the non-traumatized leg contains only 2.8 to 5.5 per cent of the total extracellular fluid.

In all the dogs there was evidence of a decrease in water content of both skin and muscle in non-traumatized areas. The average change in water content of chest skin was 4 to 5 per cent and in abdominal muscle, 2 per cent. By using Skelton's table of percentage weight of organs in relation to body weight (18), we have calculated the fluid shift from skin and muscle to be 100 to 150 cc. in the different dogs. Except for one instance these values agree with those in Table 3, column c, which represent the water found in the traumatized area in excess of that accounted for by loss from the circulating blood volume. In our opinion

this represents the transfer of tissue fluid from uninjured areas to the blood from which it is subsequently lost to the traumatized region.

Potassium analyses of the traumatized and the non-traumatized legs were included in order to determine whether the injury was sufficient to cause the

TABLE 2

*Available fluid (thiocyanate) volume in traumatic shock calculated from serum samples obtained at various times after thiocyanate injection*

DOG NUMBER.....	A	B	C	D	E	F	G
Date.....	3/4/42	3/6/42	3/11/42	3/18/42	3/25/42	4/8/42	4/15/42
Available fluid control (liters)..	2.39	2.49	2.04	1.95	2.56	4.26	2.95
Hours after injection	Available fluid (liters)						
1	1.61						
2	2.10	1.70	1.49	1.35	1.77	2.99	2.24
3	2.24	dead	1.77	1.70	2.17	3.64	2.69
4	2.41		1.97	dead	2.40	4.07	2.95
5	2.41		dead		2.40	4.26	3.12
6	dead				2.46	4.26	3.12
7					2.50	4.26	3.12
8					dead	4.26	3.12
9						dead	dead

TABLE 3

*Comparison of the increase in fluid content of the traumatized leg with the reduction in blood volume*

DOG NO.	WT.	WHOLE BLOOD LOSS		DIFFERENCE IN WATER CONTENT BETWEEN THE 2 LEGS (b)	FLUID OF TRAUMATIZED LEG IN EXCESS OF BLOODWATER LOST (c) = (b - a)	FLUID OF COLUMN (c) EXPRESSED AS PER CENT OF CONTROL BLOOD VOLUME (d)
		Cc.	Cc. H <sub>2</sub> O (a)			
	kgm.			cc.	cc.	
2	9.3	198	153	172	19	2.6
3	7.7	175	143	272	130	18.6
4	13.1	332	265	400	135	13.8
5	6.2	86	68	214	146	21.6
6	6.7	240	198	328	130	17.0
7	9.4	213	169	320	150	20.1

The dog numbers which appear in table 3 are given to individual animals which bear the same numbers when they appear in tables 4 and 5. Weight of dog 1, table 5, 9.1 kgm.

diffusion of potassium from the tissues of the traumatized area. The potassium content of the traumatized and the non-traumatized legs is shown in table 5. In 3 dogs there was no difference in the potassium content of the two legs. In the other 3 animals the loss of potassium from the traumatized leg ranged from 4 to 7 m.eq.

In traumatic shock there is no change in serum potassium until approximately

1 hour before death (19). The magnitude of the terminal rise is 5 to 7 m.eq. per liter of serum. If this rise were equally distributed throughout the extracellular fluid it would represent a total transfer from muscle tissue of 10 to 20 m.eq. of potassium, a quantity much larger than can be accounted for by the difference in potassium content of the two legs (see table 5).

TABLE 4

*A comparison of the extracellular fluid content of the traumatized with that of the non-traumatized leg*

DOG NO.	DIFFERENCE IN WATER CONTENT BETWEEN 2 LEGS (c)	DIFFERENCE IN EXTRACELLULAR WATER BETWEEN 2 LEGS (b) CALCULATED FROM		PERCENTAGE OF TOTAL EXTRACELLULAR FLUID IN THE 2 LEGS (c) (BASED ON SODIUM)	
		Sodium	Chloride	No trauma	Trauma
	cc.	cc.	cc.		
2	172	254	236	3.4	14.9
3	272	275	233	5.5	17.4
4	400	420	338	5.5	19.0
5	214	192	184	4.2	12.6
6	328	339	282	2.8	13.6
7	320	279	264	4.4	15.0

TABLE 5

*Comparison of the electrolyte content of the traumatized leg with that of the non-traumatized leg*

DOG NO.	WT. OF LEGS		TOTAL POTASSIUM		TOTAL CHLORIDE		TOTAL SODIUM	
	N. T.	T.	N. T.	T.	N. T.	T.	N. T.	T.
	gm.	gm.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.	m.eq.
1*	676		42.0		13.9		20.6	
	657		40.6		12.7		19.1	
2	787	1057	25.5	26.4	8.6	35.5	11.9	50.1
3	418	724	26.0	19.1	14.2	42.2	18.8	59.0
4	1000	1505	55.6	48.3	21.4	60.0	25.4	87.6
5	394	623	22.2	18.1	11.2	31.6	13.9	41.3
6	438	819	23.9	23.6	12.0	47.1	13.9	67.1
7	697	1081	32.0	31.0	14.8	48.3	18.9	64.1

\* These results are included to show the constancy of the electrolyte content of the 2 hind legs of a normal dog.

N.T. = non-traumatized leg. T = traumatized leg.

DISCUSSION. Although there is a three-fold increase in the time before thiocyanate reaches equilibrium in the traumatized, as compared with the normal animal, distribution is complete within 3 to 4 hours and the volume measured is the same as in the control (table 2). This result is confirmed by the similar behavior of injected sulfanilamide before and after muscle trauma (20). The thiocyanate findings are substantiated by the results of serum electrolyte studies and justify the conclusion that in traumatic shock no appreciable changes in the

volume of the extracellular compartment occur. Our results agree with those obtained by Brues et al. (21) who used both thiocyanate and radio-active sodium to measure extracellular fluid volume in shock produced by either the Blalock press or muscle ligation. Since these authors found that the average change amounted to 2.8 per cent, which is within the limits of error of the methods used, they concluded, as we have, that no change in the total extracellular volume occurs in shocked animals.

In the comparisons of the fluid shifts in uninjured and traumatized tissues the following assumptions were made: (a) that in the normal dog the electrolyte and water contents of the two hind legs are identical; (b) that the transfer of water from the non-traumatized leg is not significant; and (c) that sodium and chloride are distributed in the same volume as the thiocyanate.

Certain comments concerning each of these assumptions are pertinent:

(a) The electrolyte values obtained from the two hind legs of one normal dog are shown in table 5 (dog 1). The legs were dissected with the same technique used for the traumatized dogs and were found to weigh 676 and 657 grams. The close agreement of the electrolyte values justifies our method of comparing the two hind legs. Furthermore, Cullen and Freeman (8) have shown in 15 normal dogs that when this method of dissection is used the variation in weight, between one hind leg and the leg of the opposite side averages only 0.32 per cent of the body weight.

(b) Our method of comparing the traumatized with the non-traumatized leg might be criticised, however, on the basis that the water content of the non-traumatized leg is decreasing, whereas that of the traumatized leg is increasing. Skin and muscle samples from the non-traumatized leg were analyzed for water content before and after trauma. Calculations based on these results show that the water loss from this leg did not exceed 17 cc. in any instance. This loss, compared with the total water content of the non-traumatized leg, represents a maximum change of no more than 3.5 per cent. Since these changes are so small, it seems fair to assume that the transfer of water from the non-traumatized leg does not significantly alter our results.

(c) Although our calculations are based on the assumption that sodium and chloride are distributed in the same volume as thiocyanate, Winkler et al. (22) have shown that in dogs thiocyanate is distributed through a volume of fluid equal to 36 per cent of the body weight, whereas radioactive sodium and chloride are distributed in a volume equal to 28 and 25 per cent, respectively. Their results would indicate that the total amount of sodium and chloride in the traumatized leg should represent a larger percentage of the total sodium or chloride compartments than of the thiocyanate compartment. However, the findings of Winkler et al. are not in agreement with those of Kaltreider and his collaborators (23) who report that in man thiocyanate and radioactive sodium are both distributed in fluid volumes representing 25 per cent of the body weight. A study of the data of Brues et al. (21) in the shocked dog shows no consistent differences between the extracellular volume measured by radioactive sodium and that measured by thiocyanate. While the size of the extracellular com-

partment is controversial, any correction made on the basis of Winkler's findings would only serve to magnify our values for the percentage of extracellular fluid transferred to the traumatized leg.

The potassium data are of considerable interest for they show that there is no significant difference between the potassium contents of traumatized and non-traumatized legs. This must mean that the increase in the extracellular fluid volume of the traumatized leg is not accompanied by a decrease in the intracellular volume, but is the result of the addition of blood and extracellular fluid from other regions to the original fluid volume of the leg.

In trauma as in hemorrhage sufficient amounts of fluid are present in the extracellular compartment to restore blood volume. According to Walcott (4) the amount of fluid available after hemorrhage amounts to 10.7 per cent of the original blood volume. Our results suggest that a volume of fluid equivalent to about 15 per cent of the original blood volume can be made available for dilution of the circulating blood in the shock produced by muscle trauma (see column d, table 3). We have shown that this fluid, instead of remaining in the circulation "leaks" into the traumatized area. Although the total extracellular fluid volume does not change, the extracellular fluid of the traumatized region increases. From this one might expect that intravenously injected 0.9 per cent saline would enter traumatized regions, being limited only by increasing tissue tension. Indeed, this phenomenon is shown in the experiments of Nickerson (5) who found increases in the volume of the traumatized legs of 2 dogs equivalent to 50 and 100 per cent respectively, of 100 cc. of injected saline. Other investigators (25, 26) have reported that larger amounts of saline will cause clinical improvement and an increase in blood volume in shocked dogs. Presumably the increased tissue tension in the traumatized and other areas limits the loss of injected fluid thus permitting some fluid retention in the circulation.

#### CONCLUSIONS

The total volume of extracellular fluid is essentially unchanged in shock produced by muscle trauma. This conclusion is based on both thiocyanate and electrolyte studies.

Our results show that the traumatized area contains more fluid than can be accounted for by blood loss alone. Electrolyte and water analyses of the traumatized and the non-traumatized legs show that this fluid is contributed by the extracellular compartment. The traumatized leg contains 13 to 19 per cent of the total extracellular volume, whereas that of the corresponding non-traumatized leg contains only 3 to 6 per cent. Skin and muscle samples of uninjured areas of the body show a concurrent water loss approximately equal to the gain in the traumatized region.

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# ON THE QUANTITATIVE RELATIONSHIP BETWEEN CALCIUM AND PROTHROMBIN

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Although the indispensability of calcium in the coagulation of the blood was established in 1890 by the classical work of Arthus and Pagès (1), no accurate quantitative studies of calcium in the clotting mechanism were reported until the work of Ransmeier and McLean (2) appeared in 1938. These authors studied the effect of the concentration of calcium on the coagulation of citrated plasma. They made the significant observation that the minimal  $\text{Ca}^{++}$  concentration at which diluted citrated plasma coagulated was considerably higher for human than for dog plasma. The same year Steward and Pohle (3) found that the prothrombin time as determined by the writer's (4) procedure was slightly shortened by using a calcium chloride solution of a lower concentration than was originally advocated, i.e., less than 0.025 M. The writer (5) likewise found that the prothrombin time of normal human plasma was decreased slightly with lower concentrations of calcium chloride, but that over a range of 0.025 to 0.00125 M a variation of only 1 to 1.5 seconds occurred. In studying the amount of oxalate required to inhibit completely the coagulation of blood in the presence of excess thromboplastin, the writer made the interesting observation that the decalcification of blood by sodium oxalate was relatively slow (6), in contrast to the action of sodium citrate which he found removed ionized calcium practically instantaneously (7). Recently Jaques and Dunlop (8) reported that normal dog plasma gave little variation in prothrombin time with calcium concentrations from 0.005 to 0.10 M but dicumarol plasma showed a definite optimal calcium concentration of 0.025 M and that stronger and weaker concentrations gave much longer prothrombin times. They further noted that the concentration of calcium in the blood is not optimal in dicumarol plasma. In a later work Jaques and Dunlop (9) reported that plasma treated with aluminum hydroxide showed the same sensitivity to the concentration of calcium as they observed for dicumarol plasma.

With the accumulation of evidence that prothrombin is a complex composed of several components with which calcium is associated (7), a more concise and integrated study of calcium in its quantitative relationship to the coagulation mechanism can be undertaken. Such an investigation becomes particularly propitious because of the development of two new agents: Amberlite,<sup>1</sup> a synthetic resin which as shown by Steinberg (10) quantitatively removes calcium

<sup>1</sup> Amberlite I R 100 is a phenol-formaldehyde resin which acts as a cation-exchanger. A generous supply was furnished by Dr. E. R. Mueller of Resinous Products and Chemical Co., Philadelphia.

from blood, and a silicone, Dri-Film,<sup>2</sup> which as Jaques and his associates (11) have found markedly delays the coagulation of blood when applied as a coating to the walls of the container. By means of these two agents, the optimal calcium requirements for the prothrombin time of normal human and dog blood were determined, and a quantitative study of the change in the calcium required for the prothrombin time of dicumarized plasma was made. Further information concerning the relationship of calcium to components A and B of prothrombin was obtained.

**METHODS.** *Determination of prothrombin time.* The procedure as outlined by the writer (12) was employed. Great care was exercised in timing exactly the formation of the clot. This required accurate measuring and mixing of the plasma, thromboplastin and calcium chloride solutions; incubation in a water bath kept at 37 to 38°C.; and a uniform gentle tilting of the test tube to detect the incipient formation of the fibrin web. One large lot of thromboplastin prepared from rabbit brain dehydrated with acetone and preserved in evacuated ampules was used in the investigation. It conformed to the standards of maximum activity (6 sec. for dog plasma and 11 to 12½ sec. for human plasma) which the writer considers is the only type of preparation suitable for investigative work. One cubic centimeter serological pipettes graduated in 0.01 cc. were used. For convenience they were cut to 170 mm. lengths. The calibrations of the pipettes used for measuring the calcium chloride solutions were checked with mercury. The solutions of calcium chloride were made from a stock solution of 0.1 M, which was prepared by dissolving the calculated amount of chemically pure calcium carbonate in dilute hydrochloric acid and then neutralizing the slight excess of acid.

*Preparation of native plasma.* Blood was drawn using a needle and syringe coated with the silicone, Dri-Film, according to the directions of Jaques and his co-workers (11).<sup>3</sup> The blood was immediately transferred to a test tube coated with silicone and placed in an ice bath. Both the syringe and test tube were kept cold until used. After the blood was chilled, it was centrifuged and then again placed in the ice bath.

*Decalcification of plasma with Amberlite (Amberlite plasma).* The resin, Amberlite I R 100, which is of an analytical grade was used. Twenty grams of the resin were put into a cylindrical glass tube (16 mm. diameter and 25 cm. long) with a constricted end in which a plug of cotton was placed to retain the granular resin. Two hundred and fifty cubic centimeters of a 5 per cent solution of sodium chloride was passed through the resin in 1 to 1.5 hours. This was followed by 50 cc. of distilled water to remove the excess sodium chloride. The resin which by this treatment was put into the sodium cycle was dried at room temperature.

<sup>2</sup> Dri-Film no. 9987 (General Electric) is methyl-chloro-silone. Mr. J. S. Hurley, Jr., of General Electric kindly supplied the material used in this research.

<sup>3</sup> In coating a container with silicone, the agent is poured or drawn in, and after the sides are covered, the excess is allowed to drain. The container is rinsed with distilled water. The methyl-chloro-silane is hydrolyzed to the silicone and hydrochloric acid. After the acid is removed by repeated washing, the container is air dried.

In a 10 cc. syringe coated with the silicone, Dri-Film, 2 grams of the resin were put and held in place by a disc of copper screen also treated with silicone. The blood obtained directly from the vein was drawn slowly through the layer of amberlite until 5 cc. were collected. In transferring the blood, it was slowly expelled to allow further contact with the resin and it was collected in a test

TABLE 1

*The effect of varying the concentration of calcium chloride on the prothrombin time of normal human and dog plasma*

CALCIUM CHLORIDE CONCENTRATION	PROTHROMBIN TIME OF PLASMA*				
	Untreated (native)	Treated with amberlite	Oxalated†	Citrated‡	
<i>M</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	
0.03	12.5	13.5	12.5	12.5	Human plasma
0.02	12	13	11.5	11	
0.01	12	12	11.5	11	
0.005	12	12	11.5	15	
0.0025	12	12	11	60	
0.0015	12	12.5	12.5		
0.00125	12	13.5	15		
0.00062	12	15	25		
0.00031	12	35	45		
0.00000†	12				
0.03	6	8	6	6	Dog plasma
0.02	6	7.5	6	6	
0.01	6	7.5	6	6	
0.005	6	7.5	6	6	
0.0025	6	7.5	6	6	
0.00125	6	7.5	6	19.5	
0.00062	6	7.5	8	38	
0.0005	6	7.5	16	55	
0.0004	6	8	20		
0.0003	6	8.5	30		
0.00015	6	11			
0.00000†	6	40			

\* For the determination of the prothrombin time of native and amberlite plasma, 0.1 cc. of plasma was mixed with 0.1 cc. of calcium chloride and 0.1 cc. thromboplastin added; for oxalated and citrated plasma, the plasma and thromboplastin were mixed before the calcium chloride was added.

† Calcium free physiological saline solution was used in place of calcium chloride.

‡ Nine volumes of blood were mixed with 1 volume of 0.1 M sodium oxalate or citrate.

tube coated with silicone. The blood was again transferred to the syringe and passed a third time through the resin. Since dog blood clots very readily, it is necessary to chill the syringe and the test tube in ice and to keep the blood in an ice bath. It was found that 2 grams of the resin are required for 5 cc. of dog blood and 1.0 to 1.5 grams for human blood. Steinberg's method (10) of mixing the blood directly with the resin in a small container failed in the hands of the writer as coagulation invariably occurred before the calcium was removed.

The minimal calcium concentration required for maximum prothrombin activity of dog and human blood. Four types of plasma were obtained and studied: 1, native or untreated; 2, amberlite, plasma decalcified with the resin; 3, oxalated, 4, citrated. The prothrombin time of the 4 types of plasma with varying concentrations of calcium chloride were determined. The results are recorded in table 1. Very little individual variations were observed in either human or canine blood. The results on rabbit blood were very similar to those obtained for dog blood, but as only a few determinations were made, the data are not recorded.

The relationship of component B to the calcium requirement for optimum prothrombin time. By treating plasma decalcified by amberlite with aluminum hydroxide, according to the writer's method (13), a product is obtained which

TABLE 2

The influence of the concentration of calcium on the prothrombin time of oxalated dog plasma (as a source of component B) diluted with plasma from which calcium had been removed with amberlite and component B taken out by means of aluminum hydroxide

CALCIUM CHLORIDE CONCENTRATION	PROTHROMBIN TIME			
	Amberlite plasma (human)	Oxalated dog plasma.1 part Alumina amberlite plasma (human) ...24 parts	Amberlite plasma (dog)	Oxalated dog plasma.1 part Alumina amberlite plasma* (dog).....24 parts
M	sec.	sec.	sec.	sec.
0.02	12.5	28	8	19
0.01	12	27	8	19
0.005	12	27	8	19
0.0025	11.5	28	8	19
0.00125	12	31	8	20
0.00062	13.5	34	8	21
0.00031	35	55	9	26

\* The plasma obtained from blood which was decalcified with amberlite and treated with aluminum hydroxide to remove component B.

presumably lacks only component B of prothrombin and calcium. By adding to this a small quantity of oxalated dog plasma which has a high concentration of component B, a mixture is obtained which has sufficient component B to yield a prothrombin time suitable for accurate study yet has dominantly the composition of the amberlite plasma. The results are given in table 2.

The relationship of component A to the calcium requirement for optimum prothrombin time. When plasma decalcified with sodium oxalate is stored in a refrigerator, the prothrombin activity gradually diminishes due, as has been shown previously (7), to a disappearance of component A. In order to determine whether the diminution of component A changes the calcium requirement for optimum prothrombin activity, the effect of progressive dilutions of calcium chloride on the prothrombin time of fresh and stored oxalated plasmas were studied and the results are presented in table 3. Curiously a depression of the  $\text{Ca}^{++}$  much greater than is necessary to inhibit coagulation is required to cause

component A to diminish on storage. Thus if 9 volumes of human blood are mixed with 1 volume of 0.125 M. sodium citrate the disappearance of component A is slow, but if 0.25 M. sodium citrate is used the fall is similar to that in oxalated plasma (14). In plasma treated with amberlite the disappearance of component A is slow for apparently enough  $\text{Ca}^{++}$  remains to stabilize this factor. These results will be reported in a subsequent paper.

*Changes in the calcium requirement for maximum prothrombin activity of plasma from dogs given dicumarol.* Dogs were fed 5 mgm./kgm. of dicumarol (3,3' methylenebis (4 hydroxy-coumarin)) daily until the desired reductions in prothrombin was obtained. Four types of plasma: native, amberlite, oxalated and citrated, were obtained and the prothrombin time was determined with varying concentrations of calcium (table 4).

DISCUSSION. By removing the calcium from blood with amberlite, no excess decalcifying agent or anti-coagulant is added and therefore one can study directly

TABLE 3

*The influence of the concentration of calcium on the prothrombin time of fresh and stored human oxalated plasma*

CALCIUM CHLORIDE CONCENTRATION	PROTHROMBIN TIME	
	Fresh oxalated plasma	Stored oxalated plasma
M	sec.	sec.
0.03	14	26.5
0.02	12.5	26
0.01	12.5	26
0.005	12.5	25
0.0025	12	27
0.00125	13.5	35
0.00062	23	90

the effect of varying concentrations of calcium by merely adding the desired amount of calcium chloride. By this means it was found that the minimal amount of calcium that will bring about the shortest coagulation time in the presence of an excess of highly active thromboplastin is approximately 0.0012 to 0.0015 M for human and 0.0003 to 0.0004 M for dog plasma (table 1). Since the average concentration of calcium in 100 cc. of plasma is 10 mgm. or 0.0025 M, it can be seen that human plasma contains about twice as much as is needed for optimum coagulation, while dog plasma contains 6 to 8 times more than the optimum. Since it is generally assumed that one-half of the plasma calcium is combined or unionized, it appears that coagulation occurs at a calcium level definitely below the free or uncombined fraction. Coagulation of dog plasma can still occur when the calcium is reduced below 0.00004 M but at such high dilutions accurate results cannot be obtained as the reagents, particularly thromboplastin, contain a minute amount of calcium, and some doubtless remains in the plasma after treatment with amberlite. In fact, dog plasma treated with amberlite will usually clot in 30 to 40 seconds on the addition of thromboplastin

TABLE 4

*The effect of the concentration of calcium chloride on the prothrombin time of plasma from dogs given dicumarol*

CALCIUM CHLORIDE CONCENTRATION	PROTHROMBIN TIME OF PLASMA			
	Untreated (native)	Treated with amberlite	Oxalated	Citrated
<i>M</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>	<i>sec.</i>
0.03	40	55	48	42
0.02	38	50	47	37
0.01	36	50	45	37
0.005	35	50	52	83
0.0025	39	65	68	
0.00125	39	95		
0.00062	40			
0.00031	40			
0.00015	43			
0.00000	50			
0.03	34	33	34	31.5
0.02	30.5	30.5	29.5	29.5
0.01	31	30	31	34
0.005	31	30	34	68
0.0037	31.5	35	45	
0.0025	32.5	37		
0.00125	34	52		
0.00062	37	90		
0.00031	39			
0.00000	50			
0.03	23	22.5	22.5	23.5
0.02	22	22	23	22
0.01	23	22.5	24	22
0.005	23	23	30	45
0.0025	25	29	39	
0.00125	28	37	60	
0.00000	30			
0.03	18.5	17	17	16
0.02	17.5	17	17	16
0.01	17.5	17	18	20
0.005	17.5	17.5	20.5	35
0.0025	18	18.5	27	
0.00125	17.5	24	43	
0.00062	17.5	40		
0.00000	17.5			
0.03	12	11	11	11
0.02	11	10	10.5	11
0.01	11	10	11	10.5
0.005	11	10.5	11.5	14
0.0025	11	11	14	40
0.00125	11	11.5	25	
0.00062	11	14	95	
0.00000	11.5			

alone, whereas human plasma which requires a higher concentration of calcium will not coagulate. Nevertheless it is certain that the amount of calcium required for coagulation is exceedingly small.

The results in table 1 establish unequivocally that normal dog plasma requires less calcium than does human plasma. This is in agreement with the finding of Ransmeier and McLean that the minimal  $\text{Ca}^{++}$  concentration at which diluted citrated plasma coagulates is higher for human than for dog plasma. The findings are also in accord with the writer's earlier observation that less sodium citrate is needed to suppress the coagulation of human than of rabbit blood, since the latter has practically the same prothrombin activity as dog blood.

The exact rôle of calcium in the coagulation mechanism still remains uncertain. In previous studies the writer (7) presented evidence to show that the prothrombin is a complex consisting of calcium and two components, A and B. If one postulates that prothrombin contains calcium, then it follows that dog plasma should require more calcium than human plasma since it contains a much higher concentration of components A and B, but experimentally the reverse is found. A somewhat different explanation for the position of calcium in the clotting reaction becomes necessary.

It will be observed from the results in table 1 that a fixed minimum concentration of calcium will give the shortest prothrombin time, or produces maximum prothrombin activity. Beyond this concentration no further increase in coagulability occurs even though the calcium may be increased 50 times above the critical level in the case of dog plasma. At high concentrations of calcium, the prothrombin time becomes prolonged, but this may not necessarily be related to prothrombin activity, since the stability of fibrinogen is increased by elevated concentrations of anions (15). The behavior of calcium is best explained by assuming that plasma contains an agent which may be designated as the calcium co-factor. It requires for its saturation an amount of calcium which corresponds to the minimum concentration of  $\text{Ca}^{++}$  that produces maximum prothrombin activity. One may conjecture that the activation of prothrombin by thromboplastin is mediated through this co-factor and calcium.

With this concept, a better understanding of the prothrombin time is possible. This determination is basically a measurement of the time required for the formation of a visible clot, and this depends solely on the concentration of thrombin. The prothrombin time therefore actually measures the time required for the production of sufficient thrombin to convert enough fibrinogen to yield a macroscopic amount of fibrin. The higher the concentration of prothrombin, the faster and greater the production of thrombin, and therefore the shorter the prothrombin time. The concentration of prothrombin includes both components A and B. An alteration of the concentration of either changes the prothrombin time. Calcium, on the contrary, acts as a constant provided the concentration is maintained above the critical level (0.0015 M for normal humans and 0.0004 M. for normal dog plasma). Thromboplastin likewise behaves as a constant if a sufficient amount is present to meet the minimum effective concentration. Any amount above this has no further accelerating



action. These facts are utilized in the determination of prothrombin by the one-stage method, for a fixed quantity of calcium chloride (0.02 M) which is definitely above the minimum amount required, and an excess of thromboplastin are added to the plasma.

The concentration of neither components A nor B appears to have a direct influence on the calcium requirement for optimum prothrombin activity. The results recorded in table 2 indicate that component B does not determine the critical calcium level. By passing freshly drawn blood through amberlite and treating the resulting plasma with aluminum hydroxide, a plasma is obtained which lacks calcium and component B, but is otherwise not fundamentally altered. If to such a plasma a small amount of dog plasma is added which contains a high concentration of component B, a mixture is obtained in which the prothrombin time is essentially dependent on the concentration of component B of the added dog plasma, but the calcium requirement remains that of the treated plasma used as diluent. It will be observed that when the dilution was made with human plasma the minimum effective concentration of calcium chloride is approximately 0.0012–0.0015 M, the same as for normal human plasma, and 0.0003–0.0005 M when dog plasma treated with amberlite and aluminum hydroxide is employed. Since the concentration of component B remains the same whether the diluent is human or dog plasma, the difference in the calcium requirement must depend on some factor present in the plasma.

Although the prothrombin time of stored oxalated human plasma is greatly increased, no significant change occurs in the minimum concentration of calcium required for optimum prothrombin time. Since the fall of prothrombin activity in stored plasma is due to a decrease of component A, it can be concluded that this factor also does not directly influence the calcium requirement for prothrombin activity. Component A is, however, influenced by calcium since it apparently becomes unstable and loses activity if the concentration of calcium ions is markedly depressed as by excess oxalate ions. In plasma decalcified with 0.1 M sodium citrate or with amberlite little loss of prothrombic activity (i.e., loss of component A) occurs on storage, and this is due very likely to an insufficient depression of calcium ions, especially since a decrease of prothrombin occurs if the concentration of citrate is increased.

It will be observed on examining table 1 that the prothrombin times of oxalated and amberlite plasma, after the addition of varying strengths of calcium chloride solutions, are similar until dilution becomes relatively high, whereas the prothrombin time of citrated plasma becomes delayed when the calcium chloride concentration is below 0.005 M for human and 0.0025 M for dog plasma. These results can readily be explained. The average concentration of calcium in blood is 6 mgm. per 100 cc. which is 0.0015 M. The addition of 1 volume of 0.1 M sodium oxalate or sodium citrate to 9 volumes of blood leaves an excess of 0.0085 M sodium oxalate or citrate. The addition of 0.0085 M calcium chloride should remove exactly the excess decalcifying agent. In the case of citrated human blood, the addition of 0.01 M calcium chloride still yields the optimum prothrombin time of 11 seconds whereas a delay occurs when 0.005 M is added. When oxalated

human blood is used, no delay of the prothrombin time is observed until the concentration of calcium chloride is 0.0015 M or lower. Since sodium citrate very quickly removes ionic calcium, as the writer has shown, a delay of the prothrombin time occurs when the amount of calcium added is insufficient to leave an excess.

The removal of ionized calcium by sodium oxalate, on the contrary, is relatively slow especially as compared to the speed of the reaction of calcium in the conversion of prothrombin to thrombin. Therefore on adding calcium chloride even in amounts much below that needed to leave an excess of calcium ions, a normal prothrombin time is obtained, because the participation of calcium in the coagulation reaction is completed before the removal of sufficient calcium as insoluble calcium oxalate has occurred. If, however, the prothrombin time is prolonged, the removal of calcium as calcium oxalate occurs before the coagulation reaction is completed. Obviously, more calcium chloride must be added to a plasma with a delayed prothrombin time in order to insure an excess of calcium ions after the oxalate has been precipitated. The true calcium requirement can therefore be determined only with plasma decalcified by means of amberlite. This explains why Jaques and Dunlop found only one concentration of calcium chloride (0.025 M) optimum for dicumarol plasma instead of a range of values down to concentrations as low as 0.005 to 0.0025 M, as found in this study. They, moreover, obtained for some unexplainable reason, a prothrombin time of 20 seconds for normal dog plasma instead of 6 seconds which is consistently obtainable if potent thromboplastin is used.

The fact that the speed of the reaction with which calcium participates in coagulation is of approximately the same magnitude as the reaction with which sodium citrate removes ionized calcium but is much faster than the precipitation of calcium as the oxalate salt, has practical significance. Obviously sodium oxalate is the more suitable reagent for the determination of the prothrombin time since it permits a wider range of calcium concentrations without altering the speed of coagulation than if sodium citrate were used as the decalcifying agent.

The plasma from which the calcium is removed with amberlite frequently shows a prothrombin time slightly longer than that of oxalated plasma. In fact, it has not been possible to prepare dog plasma with amberlite having a prothrombin time of 6 seconds. The best preparation had a prothrombin time of 6.5 seconds, but usually the values ranged from 7 to 8 seconds. The reason for this has not been found but is being investigated.

When the prothrombin is decreased by feeding dicumarol, the minimum calcium concentration required to obtain the shortest prothrombin time is definitely increased as seen from the results recorded in table 4. Whereas the critical concentration for maximum prothrombin time of normal dog plasma is approximately 0.0004 M, the minimum concentration is increased to about 0.0037 M when the prothrombin time is prolonged to 30 seconds.

In dicumarol poisoning, a decrease of component B occurs, but this factor, as has already been shown, does not determine the calcium requirement. There must nevertheless be a relationship between the decrease of component B and

the increase in the minimum concentration of calcium chloride for optimum prothrombin activity. It is not possible on the basis of present knowledge to correlate these findings, but it appears that dicumarol does more than merely cause a diminution of component B.

Of great practical importance is the finding that when the prothrombin time is over 20 seconds, the concentration of calcium in the blood is no longer adequate for optimum prothrombin activity. From table 4 it can be seen that when the prothrombin time was 22 to 23 seconds for oxalated or amberlite plasma, it was 30 seconds for native plasma, and when it was 30 seconds for oxalated or amberlite plasma it was 50 seconds for plasma having the normal content of calcium. That this discrepancy is due to insufficient calcium is proved by the fact that a prothrombin time identical to that of oxalated or amberlite plasma is obtained by adding calcium to native plasma. Jaques and Dunlop also observed that the blood prothrombin time of plasma having a markedly diminished prothrombin due to dicumarol can be shortened by adding more calcium.

Obviously a procedure such as the bedside method for prothrombin, which consists in adding freshly drawn blood to thromboplastin becomes highly inaccurate as a measure of prothrombin activity when the latter is diminished since the calcium of the blood is inadequate for optimum coagulation.

The inadequacy of blood calcium for maximum prothrombin activity in marked hypoprothrombinemia from dicumarol suggests that the increase of the coagulation time is due probably to the combined effect of the reduced prothrombin and insufficient calcium. Since the prothrombin time and coagulation time do not necessarily always run parallel, studies must be made to determine whether the coagulation time is also shortened by additional calcium. If this is established, it will be the first recognized authentic condition in which an insufficiency of calcium becomes a factor influencing the coagulation of the blood.

#### SUMMARY

By means of a phenol-formaldehyde resin, commercially known as Amberlite I R 100, which acts as a cation exchanger, the calcium of dog and human blood was removed. With the plasma thus obtained, a direct quantitative study of the effect of added calcium salts was made. The minimum concentration of calcium chloride required for maximum prothrombin activity of human plasma was found to be approximately 0.0012 M and for dog plasma 0.0004 M. Higher concentrations had no further effect on the prothrombin time except that a depressing action began when the calcium exceeded 0.02 M. The optimum or critical concentrations for both human and dog plasma are below the level of the free or ionized calcium.

Evidence was obtained that the critical calcium concentration for optimum prothrombin activity is not directly dependent upon either component A and B of prothrombin. The conjecture is made that a calcium co-factor is present in plasma which with calcium and thromboplastin becomes the activator of prothrombin.

The effect on the prothrombin time of adding varying amounts of calcium to

oxalated plasma and to plasma decalcified with amberlite is similar, whereas the prothrombin time of citrated plasma becomes altered immediately when the concentration of the added calcium approaches stoichiometrically the concentration of the citrate solution. These findings are explained by the fact that citrate promptly removes ionized calcium, while the formation of insoluble calcium oxalate is relatively slow.

After feeding dicumarol to dogs, the minimum or critical concentration of calcium required for optimum prothrombin time increases proportionately to the fall of the prothrombin. When the prothrombin time is increased over 20 seconds, the normal concentration of calcium in blood is insufficient for optimum prothrombin activity.

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# LOSS OF POTASSIUM FROM STIMULATED MUSCLES IN ADRENALECTOMIZED CATS

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Potassium metabolism is profoundly altered by the removal of the adrenal cortex. The site of this disturbance of K metabolism is not known. Harrop et al. (1934) and Harrison and Darrow (1939) believe it to be largely a failure of renal excretion of potassium.

In contrast to this view, Marenzi (1938) has shown that injected K is less readily fixed by the tissue cells in adrenalectomized animals than in normal animals, and Ingle, Wilson and Kendall (1937) have shown that the administration of adrenal cortical extract is beneficial even in adrenalectomized nephrectomized animals. Thus it is possible that the disturbance of the K metabolism is, at least partly, located in the tissue cells, and in that case adrenalectomy might be expected to affect the relation of K to muscle stimulation and contraction beyond any possible effect due to the high concentration of K in the plasma.

Somogyi and Verzar (1941) have reported that while potassium was liberated from the muscles of normal cats during contraction elicited by stimulation of the sciatic nerve, it was not released in adrenalectomized cats under identical conditions. In these experiments the same amount of work was done as was done by the normal controls. However, Jordan (1945), working on adrenalectomized rats, found that the K concentration of the muscles decreased during muscular stimulation, provided the muscle was able to do work.

An earlier paper of Verzar and Somogyi (1940) states that contracture of muscles elicited by acetylcholine is not accompanied by the liberation of K in adrenalectomized cats. On the other hand, Cicardo and Moglia (1940) have reported the liberation of K from the muscles of adrenalectomized toads when they are perfused with a solution containing acetylcholine.

Tipton (1938) has shown that the injection of cortin into normal cats tends to cause a decrease in the amount of K liberated by the contracting muscle. If this is true, it seems unlikely that a cortical deficiency would have a similar effect.

Due to the conflicting nature of the reported data, it seemed desirable to repeat the work of Somogyi and Verzar in an effort to elucidate the problem.

**METHODS.** Stock, male cats weighing from 2 to 3.5 kgm. were adrenalectomized in two operations, using 45 mgm. of Nembutal per kgm. body weight (intraperitoneally) as the anesthetic, and allowing 7 to 10 days to elapse between operations. The adrenal glands were removed, one at each operation, by lumbar incision, using as few ligatures as possible to control bleeding. At the completion of each operation, in most of the experiments, a heart puncture was done and blood was withdrawn for K analysis. These analytical results, for each

cat, were used to furnish a normal K concentration value as a base line for future comparison.

Of the nine operated animals, three were maintained after the second operation on daily, intramuscular injections of 0.5 to 2.0 mgm. of desoxycorticosterone acetate over a period of about a week. This enabled the animals to recover fully from any operative shock which might have influenced the results. Six other animals were not given the adrenal hormone but were given a single injection of 50 ml. of physiological saline solution intraperitoneally immediately following the second operation. These non-maintained cats were comparable to the animals used by Somogyi and Verzar.

Symptoms of adrenal deficiency began to appear about the second day after the second operation or after the cessation of hormone administration. The experiment was performed when the deficiency symptoms had become marked, 2 to 8 days after operation. The criteria used in determining the degree of deficiency were loss of appetite, ataxia, inactivity and lowered body temperature. When these criteria seemed inadequate or difficult of evaluation, hemoglobin concentration determinations were made on capillary blood from the ear once or twice a day, until the concentration rose by 3 to 6 grams/100 cc. The technique of muscle stimulation and blood sampling was similar to that described by Fenn et al. (1939). The cats were anesthetized with Dial in urethane (0.65 cc./kgm. body wt.) and the sciatic nerve of one leg was exposed in the sciatic notch. The hamstring branch of the nerve was severed and the remaining, peripheral end was drawn through silver ring electrodes in a glass tube. The femur was fixed in place by means of brass pins screwed into the knee joint from either side. The Achilles tendon was cut and attached by a heavy linen thread to an isometric recording lever as described by Fenn et al. (1938). This served to keep the initial tension constant at 200 grams.

A tight ligature was tied around the ankle to control bleeding. The thigh, exclusive of the femoral artery, vein and nerve, and the sciatic nerve, was also tied off by a heavy ligature in six of the experiments. In three other cases, this ligature was omitted. This ligature served to obstruct any collateral blood flow and make certain that all the blood from the lower leg returned via the femoral vein.

A cannula was inserted into the saphenous vein a few millimeters distal to its junction with the popliteal to form the femoral vein. From this point, the cannula could be pushed past the valve into the femoral vein. By obstructing the femoral vein central to this point, blood could be made to flow out through the cannula. Normal circulation could be re-established at any time by releasing the femoral vein and clamping the cannula.

Chlorazol fast pink (purified) (Modell, 1939) was used in all cases to prevent blood clotting. This dye was injected into the other saphenous vein in a 5 per cent solution, 1.5 cc./kgm. body wt. The injection was made slowly (less than 1 cc./min.) since more rapid injection tended to cause respiratory distress and vomiting.

If arterial blood samples were taken, they were drawn by syringe from the

carotid artery which was clamped off between samplings. Blood samples were put into hematocrit tubes and centrifuged within 30 minutes of the end of the experiment. Hematocrit ratios were read and the plasma was then transferred to platinum crucibles for ashing.

Intermittent, high frequency stimulation, adjusted to give a maximum response, was continued for  $1\frac{1}{2}$  to  $2\frac{1}{2}$  minutes in six instances and for 5 to 7 minutes in three others. This was long enough in all cases to obtain two blood samples. All contractions were recorded on a slowly moving kymograph and the tension-time was calculated from the average tension and the actual duration of the stimulation.

Ordinarily, at the beginning of the experiment, an arterial sample was drawn, followed by a resting, venous sample from the saphenous cannula. Immediately thereafter, stimulation was begun and two venous samples were taken during stimulation and another immediately afterwards. Other venous and arterial samples were collected 10 to 15 minutes later. Occasionally, as in cat 15, a second period of stimulation was possible. Blood flow was measured and seen to increase in each cat during the period of muscle stimulation. Plasma K was determined in duplicate for each sample by the Shohl and Bennett method as modified by Fenn et al. (1938).

At the end of most of the experiments, the gastrocnemius muscle of each leg was removed for determinations of the K and water concentrations.

**RESULTS.** The experimental results on nine adrenalectomized and two control cats are shown in table 1. As previously mentioned, of the nine adrenalectomized cats, six received no further treatment subsequent to operation, while three were maintained for several days after operation on daily intramuscular injections of desoxycorticosterone acetate. The resting, venous K concentration, the maximal K concentration, and the increment of concentration of plasma K during muscle contraction are summarized in the table and compared with those of Somogyi and Verzar. Graphs of the control animals and three representative adrenalectomized animals are pictured in figure 1 so that the K increase may be more easily visualized.

The average increase of K concentration on muscle stimulation (2.9 mM K/liter) in the venous plasma of all adrenal deficient cats is seen to be in excellent agreement with that of the two controls as well as with the controls of Somogyi and Verzar. Although the resting venous plasma K concentration in the control cats is only 1 mM/liter lower than that average in the deficient cats, the experimental animals were not believed to be in any less advanced stage of adrenal deficiency than those of the other authors. Several other cats were so deficient that they died before the muscle could be stimulated and it is interesting to note that two of these animals had venous plasma K concentrations of not more than 6 mM/l. It has been pointed out by Cattell and Civin (1938) and by Zwemer and Truszkowski (1937) that in a series of normal, resting, anesthetized cats, the venous plasma K concentration is, on the average, 4.6 and 4.9 mM/liter, respectively, but that this may vary from 4 to 5.9 mM/liter. Zwemer and Truszkowski found that in adrenal deficient cats the plasma K concen-

TABLE 1

*The effect of adrenalectomy on the K shift during muscle stimulation*

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
CAT NO.	WT.	TIME	DAYS	TENSION-TIME		TENSION TIME PER GRAM	MUSCLE PER 100 GM. DRY		PLASMA K				
				Initial	Total		K	H <sub>2</sub> O	Normal	Art.	Venous		
											Rest	Stim.	ΔK
	kgm.	min.		gm.-min.	gm.-min.	gm.-min.	mM	ml.	mM/l.	mM/l.	mM/l.	mM/l.	mM/l.
1	1.6	2.5		664	2827				5.6	5.6	5.6	9.3	3.7
5	1.9	3.5		881	1618				5.5	5.0	5.4	7.6	2.2
12	3.4	2.0	8	202	449	14	-0.6	-2	5.0	8.0	7.3	10.2	2.9
13	3.5	2.5	4	566	1369	41	-3.7	-39	4.9	6.9	7.4	8.2	0.8
16	3.2	2.5	4	459	1059	37	-6.7	-5	4.6	6.9	7.7	10.0	2.3
15	3.6	2.75	2	674	1622	48	-1.6	-34	4.5	5.1	5.6	9.3	3.7
15	3.6	1.25	2	451	869	26	-1.6	-24	4.5	6.7	6.0	13.0	7.0
17	2.2	3.25	2.5	663	936	49	-3.6	-5	4.1	6.4	5.5	6.2	0.7
18	2.7	1.75	3	318	629	22	-2.7	-54	4.0	4.5	6.0	11.5	5.5
23	2.5	5.0	4								7.9	9.1	1.2
24	2.5	7.0	4								9.7	12.1	2.4
26	2.2	5.0	5								7.1	9.2	2.1
Averages													
Control cats. ....									5.6	5.3	5.5	8.5	3.0
Adrenalectomized cats. ....									4.5	6.3	7.0	9.9	2.9
Averages of Somogyi and Verzar*													
Control cats. ....											4.3	7.3	3.0
Adrenalectomized cats. ....											7.2	7.7	0.5

\* Data of two control and nine adrenalectomized cats, compared with the results of Somogyi and Verzar.

Column (1)—cat no.; (2)—weight of cat in kgm.; (3)—minutes of stimulation; (4)—time between the removal of the second gland and the experimental procedures; (5)—initial tension-time, expressed as gram-minutes of tension-time produced in the first half minute of contraction; (6)—total tension-time; (7)—total tension-time per gram wet weight of stimulated muscle; (8)—mM. of K lost during stimulation per 100 grams dry weight (mM.K/100 grams dry weight of stimulated muscle minus mM.K/100 gram dry weight of unstimulated muscle); (9)—difference in water content between stimulated and unstimulated muscle, expressed as cubic centimeters of H<sub>2</sub>O per 100 grams dry weight; (10, 11, 12 and 13)—mM. K/liter of plasma; (10)—heart puncture sample at time of second operation; (11)—resting arterial sample (carotid); (12)—resting venous sample; (13)—stimulated venous sample; (14)—difference between mM. K of stimulated and resting venous samples.

First group, controls; second group, maintained adrenalectomized; third group, non-maintained adrenalectomized.

tration rises with every day after operation, but that "the level may be normal or even subnormal during certain phases" of the adrenal insufficiency syndrome, and that "the plasma K values at any one time are not always a criterion of the degree of insufficiency present".



If only the average increment of plasma K concentration of the three maintained cats be considered, on the basis that these three were more completely controlled than the others, it is found that the average is 2.0 mM K/ liter. This is probably not significantly different from the average of the controls. As the tension-time for these animals is also lower than that of the controls (959 grams-min. as opposed to 2222 gram-min.), it is possible that we should expect a slightly smaller rise of the plasma K concentration, although a quantitative relation between work and K increment has never been conclusively demonstrated.

Of the nine deficient cats, there were two (nos. 13 and 17) which showed an increase of plasma K concentration of only 0.8 mM/ liter. The rise of K concentration in these two cats seems to parallel the general results obtained by

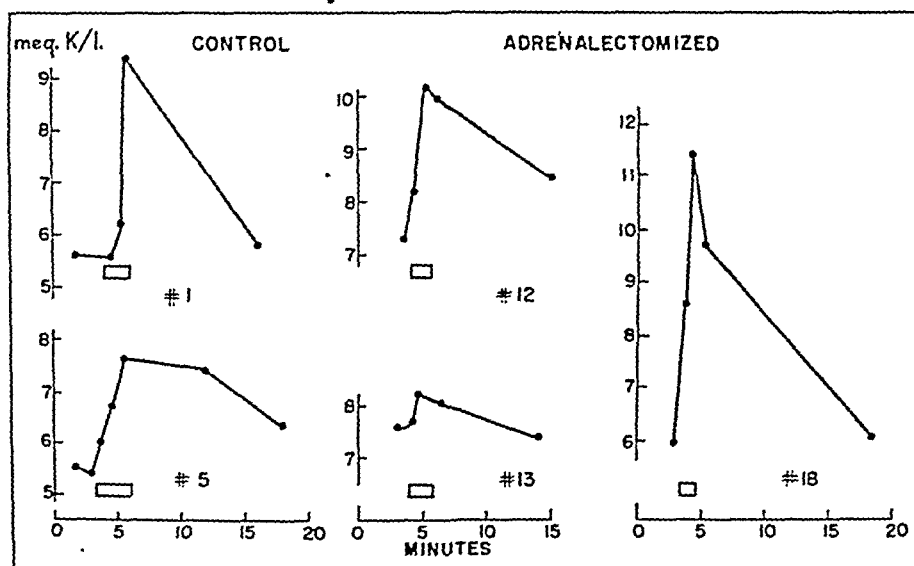


Fig. 1. Increases in concentration of potassium in the venous blood resulting from stimulation of the motor nerve. Blocks indicate periods of stimulation. No significant difference is evident between the control and the adrenalectomized animals.

Somogyi and Verzar, who found an average increment of only 0.5 mM K/liter for all their adrenalectomized cats. Since one of the cats (no. 13) was maintained and the other (no. 17) was not maintained, the possibility of operative shock does not appear to be a factor. While the authors are unable to account for this variability in their results, they feel certain that these two animals were no more deficient than the other seven in which the loss of K was more normal. It will be noted that the resting K level in these animals was not exceptionally high.

There is no apparent correlation of rise of plasma K concentration and loss of muscle K, as measured, since the plasma K concentration was always back almost to resting concentration 10 to 15 minutes before the muscles were sampled, with the exception of cat 17. The fact that there is some evidence of diminished muscle K in some instances merely indicates incomplete recovery after stimulation. When there is a loss of muscle K there is a notable gain in muscle water.

In this small series of animals, no correlation appears to exist between the rise of plasma K concentration and the muscle tension-time.

The experiments reported in this paper showed normal, large losses of K into the blood on muscle stimulation in adrenalectomized cats in contrast to the very small losses reported by Somogyi and Verzar. The authors are quite certain that the difference is not due to any less complete deficiency in their animals. Deficiency symptoms were always very marked, and, in an effort to allow adequate time to elapse after operation, the animals were left so long that several of them were unable to withstand the experimental procedures and died before blood samples could be obtained. Blood pressure was always very low, as shown by the low rate of blood flow, the hematocrit ratio was high, and anesthesia was produced by  $\frac{2}{3}$  of the normal dosage.

There were, however, certain minor differences in technique which might be of significance. In the first six experiments reported here, the whole thigh of the animal was tied off to block the collateral circulation and to avoid any possibility of drawing blood samples derived in any part from unstimulated muscle. It was thought that this might account for the difference in results. To check this, three other experiments were performed without this ligature. The results of these later experiments (cats 23, 24 and 26) were in complete agreement with the first six.

A second point of difference is that Somogyi and Verzar stimulated for only one minute, usually collecting only one blood sample during stimulation while in the experiments reported here, stimulation was continued for 2 to 7 minutes and two blood samples were obtained. The rise of plasma K concentration seemed to appear most clearly in the second sample following the beginning of stimulation, as the graphs in Figure 1 indicate. However, the length of time of stimulation would not seem to be the deciding factor since the authors' control experiments show the same lag in the time of increase of plasma K concentration. A careful comparison of these data with the data obtained on normal cats by Fenn (1935) showed no significant differences.

Hence, these experiments do not confirm the findings of Somogyi and Verzar. Adrenalectomy does not appear to modify the copious liberation of K from contracting muscle. The results reported here seem to be in agreement with the earlier work of Jordan (1945) and seem to indicate that the changes in potassium metabolism induced by adrenalectomy leave unchanged the K exchanges of contracting muscles.

#### SUMMARY

Adrenalectomy in cats resulted in changes of K metabolism as shown by a high concentration of K in the plasma but, in general, did not affect the release of K from stimulated muscle. Although, in two instances, abnormally small amounts of K were released, the average of 9 experiments was in agreement with the results of control experiments. These results are in contrast to those of Somogyi and Verzar (1941), who found a decrease in the liberation of K in adrenalectomized cats.

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# THE LOCATION AND SHAPE OF THE RIGHT ATRIUM<sup>1</sup>

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Values obtained in the measurement of the venous pressure always imply a zero point selected for reference. While it is generally agreed that the center of the right atrium should be designated as this reference point, considerable controversy has arisen as to the most satisfactory method of locating this point with respect to the landmarks of the chest.

The work done previously has disclosed certain differences of opinion in locating the zero level. Thus, Eyster (1) has recommended that a point  $\frac{1}{3}$  the distance dorsad from the ventral surface of the thorax at the fourth intercostal space should be employed. This was arrived at on the basis of measurements in the recumbent position in cadavers and of orthodiagraphic determinations. Lyons et al. (6) measured the location of the right atrium in cadavers and found that a point 11 cm. above the dorsal aspect of the chest corresponded to the usual location of the right atrium in the horizontal position. Richards et al. (8) concluded that the reference points of both Eyster and Lyons were applicable on the basis of venous pressure measurements made with long rubber catheters passed into the right atrium of human subjects in the horizontal supine position; the position of the catheter was located roentgenoscopically and roentgenographically. Holt (5) concluded that the reference point of Lyons was the most reliable except in thick-chested individuals. He based this conclusion on the arithmetic difference of two measurements of the venous pressure using the spine as the reference point; one measurement was made in the supine and the other in the prone horizontal position.

Aside from these static sources of error, errors may enter into the measurements of venous pressure by virtue of the variable architecture of the heart and chest in different subjects. In addition, the position of the subject may not always be the same. Thus, in orthopnea, the supine position cannot be used readily and in shock the patient may be in the Trendelenburg position. Furthermore, the shape and position of the heart may be altered in disease so that a reference point applicable to the normal heart may not be correct when disease is present.

In order to test the significance of these factors measurements were made in the dog under several conditions after visualization of the right atrium with radio-opaque material. The studies reported here were along three lines:

1. A comparison of dogs of varying size and with different shaped chests.

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2. The effect of shifting the dog's position from the supine.

3. The effect of producing a plethora and of inducing severe hemorrhage.

**METHOD.** Dogs varying in weight from 5 to 22.3 kgm., and with various depths of chests, were anesthetized with sodium pentobarbital (25 mgm./kilo intravenously). One of the external jugular veins was cannulated with a 16 to 18 gauge cannula and a rapid instillation of 10 cc. of 70 per cent Diodrast was made via this cannula in order to visualize the interior of the right atrium<sup>4</sup>. Films were taken at 76 cm. film-anode distance, 10 second exposure, 100 milliamperes and from 70 to 90 kilovolts. Left lateral views and antero-posterior views of the chest (with the central rays of the tube aimed at the approximate level of the heart) were taken in twelve dogs held in the supine position.

In all dogs a second exposure was made upon the film of a grid measuring device<sup>5</sup>. For this purpose, in six dogs lateral views were taken, the film was left in place, the dog removed and the grid placed in a vertical plane on the line upon which the dog's spine had previously rested (a distance of 14 cm. from the film). Then a second exposure was made, printing the grid marks upon the film. From this film, the center of the right atrium could be located in the dorso-ventral plane. In six other dogs the procedure was reversed. With the dogs in the supine position, antero-posterior views were taken first, from which the vertical plane in which the center of the right atrium is located could be determined. Next, lateral views were taken and the grid placed for the second exposure in the vertical plane so determined.

The films showed good contrast for the Diodrast-filled superior vena cava, right atrium and azygos veins. The inferior vena cava was rarely filled. Occasionally the thickness of the walls of the right atrium could be made out since the Diodrast outlined the endocardial limits and the lungs afforded good contrast with the epicardial limit. The bony structures were well made out and soft tissue detail was adequate. The films showed clearly the equidistant dots of the grid. Such films (cf. figs. 1 and 2) permitted ready measurement of the dimensions of the right atrium in the dorso-ventral, cephalo-caudad and side-to-side planes. From these measurements the mid-point of the right atrium was determined and marked on the lateral and antero-posterior films. In each direction, the mid-point was considered to be the half-way distance between the outermost margins of the right atrium. In the dorso-ventral diameter these two points were respectively the most ventral point and the dorsal point where the superior vena cava entered the atrium. The distance of the mid-point in the dorso-ventral plane from the back (including the soft tissue) was measured and computed as a percentage of the dorso-ventral diameter of the chest in this

<sup>4</sup> We are indebted to the Winthrop Chemical Co. for the Diodrast used in these studies and to Dr. R. Arens of the X-Ray Department for the use of his facilities in obtaining the roentgenograms.

<sup>5</sup> This measuring device consisted of a grid made from a thin brass plate perforated by small holes which marked the corners of squares having sides  $\frac{1}{2}$  inch in length. Due to the divergence of the roentgen rays, the grid marks on the film were more than  $\frac{1}{2}$  inch apart, but the horizontal and vertical distances between the grid marks were all the same.

plane. Likewise, the distance of the cephalo-caudad mid-point from the dome of the diaphragm and its relation to the segments of the sternum were determined.

Certain possible sources of error in such measurements were recognized and efforts made to avoid them. No error was introduced if the film-anode distance was not exactly 76 cm. in all of the dogs or if the central rays of the tube were not



Fig. 1. Reproduction of lateral x-ray of Diodrast filled heart with grid-points superimposed. Discussed in text.

identically directed in all of the animals as long as the position of the tube and film remained unchanged when the two exposures of the film were made. This is due to the fact that the grid marks would be distorted in identical manner and therefore would automatically correct such distortions, since all distances were measured in terms of distances between grid marks. This does not exclude errors which arise if the dog and/or the grid are not perfectly vertical in taking

the left lateral exposures or horizontal in antero-posterior exposures. Great care was therefore used in positioning of the dog and grid.

An error is introduced when the grid is not placed in the plane of the mid-point of the atrium since at 76 mm. the x-rays are divergent. The greater the distance between the plane of the grid and that of the atrial mid-point, the larger is the



Fig. 2. Reproduction of antero-posterior x-ray of Diodrast filled right heart. Discussed in text.

error. Since  $\frac{1}{2}$  of the dogs had the grid placed in the plane of the spine (which is to the left of the right atrium) the distortion so produced was measured in two dogs. Two left lateral films were made of each dog with the dog so placed that the spine rested on a line 15 cm. from the film. In superimposing the grid markings, the grid was placed in one instance in the plane of the spine and in the other in the plane of the mid-point of the right atrium actually determined previously by an antero-posterior film. The data are summarized in table 1 and in both dogs there was a difference of 2.5 per cent. This error is eliminated when the

distance of the mid-point from the back is calculated as a percentage of the dorso-ventral diameter.

RESULTS. 1. *A comparison of dogs of different sizes and various shaped chests.* The location of the right atrium with respect to the heart is shown in figures 1 and 2. It is located ventrally and at the right lateral cephalad portion of the heart. It is a smooth or slightly irregular cavity. In table 2 are summarized the pertinent data on the location of the mid-point of the right atrium in dogs in the horizontal recumbent position. The mid-point lies below the third or fourth sternal segment (counting from the xiphoid), the most common location being the bottom of the fourth segment and at the level between the fourth intercostal space and fifth rib dorsally. The mid-point is located from 1.3 cm. to 2.6 cm. to the right of the mid-line, the average distance being 1.85 cm. The average distance of the mid-point cephalad to the dome of the diaphragm is 5.4 cm., ranging from 4.5 to 6.1 cm.; this is a fairly fixed orientation.

TABLE 1

*Effect of grid position on the measurement of grid dots on film*

DOG NO.	EXTERNAL CHEST DIAMETER		DOG WEIGHT	GRID LOCATION		GRID MEASUREMENT			
	Dorso-ventral	Lateral		From film	To right of midline*	Actual	On film	Distortion	Difference
	cm.	cm.	kilo	cm.	cm.	cm.	cm.	%	%
1	18	10.5	11.4	16.5	2.5	15.2	19.4	27.6	2.5
				14.0	0	15.2	18.9	24.4	
2	17	11.5	11.4	16.5	2.5	15.2	19.4	27.6	2.5
				14.0	0	15.2	18.9	24.4	

\* This was the plane of the mid-point of the right auricle.

The distance of the mid-point from the back was found to be more accurately expressed as a percentage of the external chest diameter than in centimeters from the back. By the former measurement, it constituted 67 to 72 per cent of the back to front distance and averaged 69 per cent. By the latter, it was 8.7 to 14.0 cm. from the back, averaging 11.0 cm. The range of variation is considerably less by the percentage measurement than by the actual distance from the back. This favors Eyster's method in preference to that of Lyons.

Some of this difference may be an error due to the placement of the grid discussed above under methods. But even in the six dogs in which the grid was placed in the vertical plane of the mid-point the percentage measurement was the more constant, viz., the range being 67 to 72 per cent as against 8.7 cm. and 12.2 cm. This is not surprising considering the range of chest depth encountered in these dogs, viz., 12.8 to 20.8 cm. In man the chest depth range is normally less, hence the Lyons method would be applicable. But cases of funnel and barrel chest are encountered and it would appear to be better to use a percentage method in obtaining the level of the mid-point of the right atrium in the dorso-ventral diameter.



TABLE 2  
*Location of mid-point of right atrium*

DOG NO.	WEIGHT	EXTERNAL CHEST DIAMETER		RELATION OF MID-POINT OF RIGHT ATRIUM TO				RATIO OF MID-POINT OF RIGHT ATRIUM TO BACK TO EXTERNAL DORSO-VENTRAL CHEST DIAMETER
		Dorso-ventral	Lateral	Diaphragm, cephalad to	Sternal segments*, below	Midline (spine), right of	Back, ventrad to	
	kgm.	cm.	cm.	cm.		cm.	cm.	%
1	10.0	15.9	10.5	5.1	bottom 4th		10.8	67.9
2	22.3	20.8	15.6	6.0	bottom 4th		14.0	67.3
3	9.1	14.7		6.1	top 3d		10.2	69.4
4	15.9	17.9	12.8	5.1	middle 4th		12.6	70.4
5	11.8	17.1		6.0	bottom 4th		12.3	71.9
6	13.2	15.0		4.5	middle 4th		10.8	67.9
7†	5.9	12.8		5.1	bottom 4th		8.7	68.0
8†	11.4	18.0	10.5	4.5	top 3d	2.6	12.2	67.7
9†	11.4	17.0	11.5	5.7	bottom 4th	2.2	11.4	67.0
10†	8.4	15.9	11.4	5.7	bottom 4th	1.3	10.8	67.9
11†	6.4	14.0	11.5			1.3	9.5	67.9
12†	5.9	12.8	10.8	5.7	bottom 3d		9.2	71.8
Average.....				5.4			11.0	68.8

\* Measured from xyphoid.

† Grid placed in mid-point.

TABLE 3

*Effect of altering the dog's position upon the location of the mid-point of the right atrium*

DOG NO.	POSITION OF ANIMAL	RELATION OF MID-POINT OF RIGHT ATRIUM TO			
		Diaphragm, cephalad to	Sternal segments*, below	Mid-line (spine), right of	Back, ventrad to
		cm.		cm.	cm.
1	Supine horizontal	5.9	bottom of 4th		11.4
	Head-up	5.1	top of 5th		11.4
	Head-down	4.5	middle of 4th		11.4
2	Supine horizontal	7.4	top of 4th		9.8
	Head-up	7.9	bottom of 4th		9.2
	Head-down	7.5	top of 4th		10.2
	Right side	5.0		1.0	
	Prone	4.6	junction of 3d and 4th		10.2
	Left side	3.5		1.2	

\* Measured from xyphoid.

2. *The effect of shifting the dog's position from the supine.* The effect of shifting the position of the animal was studied in two dogs. The animals were rotated 90° from the supine horizontal to (1) a head-up position and (2) a head-down position. In addition, one of these dogs was rotated on its long axis from the

supine horizontal 90° to lie on its right side, another 90° to lie prone and again 90° to lie on its left side. The location of the mid-point of the right atrium was determined in the same way as before.

The results are tabulated in table 3. It was found that a change to the head-down position shifted the heart cephalad, while a change to the head-up position shifted the heart caudad. The difference in the position of the mid-point of the right atrium between the head-up and head-down positions equalled 7.6 cm. in a caudo-cephalad direction. Measuring from the caudal portion of the first sternal segment there was a shift of 3.1 cm. The relation to the diaphragm was altered but the relation to the back was not changed. Shifting the dog to either side caused the mid-point of the right atrium to shift to the other side. The relationship to the diaphragm in these two positions and in the prone position

TABLE 4

*Effect of altering the dog's position on location of the superior vena cava—right atrium junction*

DOG NO.	POSITION OF ANIMAL	RELATION OF JUNCTION OF SUPERIOR VENA CAVA—RIGHT ATRIUM TO			
		Diaphragm, cephalad to	Sternal segments, below	Back, ventrad to	Mid-line (spine), right of
		cm.		cm.	
1	Supine horizontal	6.8	bottom of 3d	7.7	
	Head-up	7.3	middle of 4th	7.7	
	Head-down	5.7	bottom of 3d	7.7	
2	Supine horizontal	6.5	bottom of 3d	9.0	
	Head-up	8.5	bottom of 4th	9.0	
	Head-down	7.0	middle of 3d	9.2	
	Right side	6.0			0.8
	Prone	6.2		9.0	
	Left side	5.3			1.0

was altered. However, the relation of the mid-point of the right atrium to the back was not affected in going from the supine to the prone position.

It is thus apparent that shifting the animal's position alters the position of the heart except that it does not affect the distance between the mid-point of the right atrium and the back. The relation of this point to the diaphragm is variable when the position of the dog is altered and is not predictable. A shift of the heart in the direction in which gravity operates occurred in all the shifts excepting between the supine and prone horizontal positions.

In contrast with the shift of the mid-point of the right atrium there is a fixation of the point at which the venae cavae enter the right atrium. This is shown in table 4, in which data similar to those in table 3, are assembled for this point of the right atrium.

3. *The effect of plethora and hemorrhage.* Three dogs were used in this study. In two, visualization in the supine position and measurements were carried out as above, although no antero-posterior views were made. After the control

film was taken, 500 to 700 cc. of blood was rapidly infused intravenously and another visualization of the right atrium made. The dogs were then bled rapidly via the carotid artery and during the bleeding films of the visualized right atrium were taken until the animal succumbed.

In the third dog, lead tags were used to outline the atrial limits. These were sutured to the junction of the superior vena cava and the right atrium, to the ventral junction of the right atrium and the ventricle, and at two points on the anterior border of the right atrium. The chest was then closed, the pneumothorax relieved and natural breathing established. Then the procedures outlined in the other two dogs were repeated without the use of Diodrast.

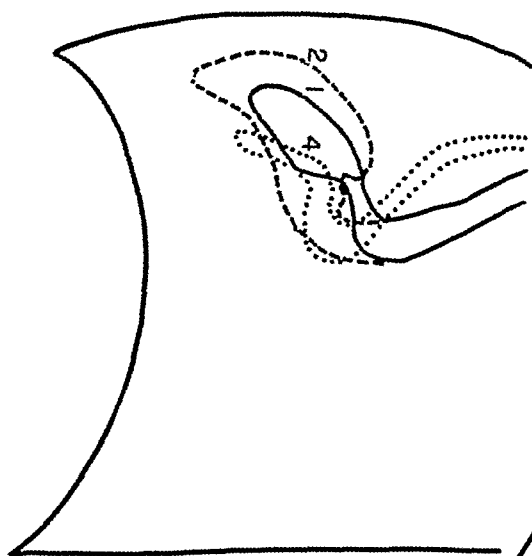


Fig. 3

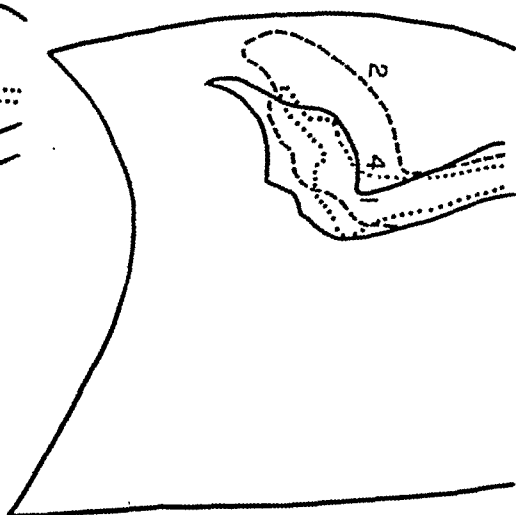


Fig. 4

Fig. 3. The positions of the superior vena cava and right atrium are indicated in the control by heavy unbroken lines (1), after rapid intravenous injection of 330 cc. of blood by dashed lines (2) and after bleeding a total of 750 cc. of blood by dotted lines (4). Discussed in text, data shown in table 5.

Fig. 4. The position of the superior vena cava and right atrium are indicated in the control by heavy unbroken lines (1), following rapid injection of 450 cc. of blood into the external jugular vein by dashed lines (2) and after bleeding a total of 500 cc. rapidly by dotted lines (4). Discussed in text, data shown in table 6.

The results in the first two dogs are depicted in figures 3 and 4 by superimposed line sketches of the three films, one control, the other two respectively the plethora stage and a stage after bleeding. The superimposition was made so that the back line of the chest, the diaphragm and the anterior line of the chest all coincided. The data on the position of the mid-point of the right atrium are shown in tables 5 and 6. The evidence from the third dog fitted in with these data.

Sudden plethora caused the right atrium to balloon out. There was practically no displacement of the dorsal portion of the right atrium but considerable outward movement of its ventral and cephalad surfaces. The mid-point consequently shifted ventrally and cephalad. These directions of shift were deter-

mined by the paravertebral muscle mass and the ventricles. The point at which the superior vena cava enters the right atrium remained fixed and showed no shift either in the dorso-ventrad or cephalo-caudad directions.

Exsanguination produced opposite effects. The right atrium shrunk in size and became slit-like. There was practically no displacement of the dorsal

TABLE 5

*Effect of plethora and hemorrhage on the location of the mid-point of the right atrium (dog A)*

STATE OF DOG	RELATION OF THE MID-POINT OF RIGHT ATRIUM TO BACK, VENTRAD TO	RATIO OF MID-POINT OF RIGHT ATRIUM TO BACK TO EXTERNAL DORSO-VENTRAL CHEST DIAMETER	REMARKS
	cm.	%	
Control	10.0	65.0	
After injection of 330 cc. blood	10.8	69.7	Right atrium ballooned out and superior vena cava dips before entering it
After rapid bleeding of 500 cc.	10.1	65.2	Right atrium slit like
After rapid bleeding of 160 cc. more. Dog tachypneic	10.1	65.2	Right atrium slit like
After rapid bleeding of 90 cc. more. Dog moribund	10.25	65.4	Right atrium slit like

TABLE 6

*Effect of plethora and hemorrhage on the location of the mid-point of the right atrium (dog B)*

STATE OF DOG	RELATION OF THE MID-POINT OF RIGHT ATRIUM TO BACK, VENTRAD TO	RATIO OF MID-POINT OF RIGHT ATRIUM TO BACK TO EXTERNAL DORSO-VENTRAL CHEST DIAMETER	REMARKS
	cm.	%	
Control	10.5	65.5	
After injection of 450 cc. blood	11.0	68.0	Right atrium balloons out. Superior vena cava dips before entering it
After rapid bleeding of 250 cc.	10.1	63.2	Right atrium slit-like
After rapid bleeding of 250 cc. more	10.1	63.2	Right atrium slit-like

aspect of the right atrium but considerable dorsal movement of its ventral portion. Its dorso-ventrad and cephalo-caudad diameters decreased, the latter more than the former. The mid-point of the atrium shifted little from the control. However, in one case there was a caudad and dorsad displacement.

The overall shift in the mid-point was of the order of 1 cm. between plethora and exsanguination, a surprisingly small difference.

DISCUSSION. The results obtained in this study show that while there is a

variability in the position of the mid-point of the right atrium, it is not so great as to nullify its utility as a zero level reference point in venous pressure measurements. The relative constancy of the location of the mid-point of the right atrium within the chest applies in a single animal through 360° of rotation on either a transverse or longitudinal axis. When the blood volume is altered from states of plethora to exsanguination, this is also true. A similar relative constancy for this mid-point is present when dogs of various weights and chest shapes are compared. It was found, however, that a value of 70 per cent of the dorso-ventral chest diameter above the back locates the mid-point of the right atrium more accurately than a fixed distance above the back.

Nevertheless our results indicate that errors of as much as 1 cm. in venous pressure readings may arise from improper location of the zero reference point. This fact should be realized in clinical venous pressure measurements especially in patients with unusual chest configuration, with marked displacements of the heart and with conditions leading to marked distention or contraction of the right atrium.

These studies suggest that in the semi-upright or Trendelenburg positions, the dome of the liver as determined by percussion can be used to follow the shifts of the mid-point of the right atrium.

#### SUMMARY

1. A method of measuring the right atrium of the living dog is described. It depends upon the roentgenogram of the Diodrast-filled chamber calibrated by means of a grid. In this manner the size, position and contour of the right atrium were determined.

2. The mid-point of the right atrium was found to be about  $7/10$  of the total antero-posterior distance from the sternum to the back of the chest. This was a constant finding in the several animals and during rotations of the animal.

3. The mid-point of the right atrium was found to vary in its relation to other points.

4. Sudden plethora and exsanguination changed the shape of the right atrium, and the position of the mid-point in the dorso-ventral diameter shifted as much as 1 cm. The position of the dorsal aspect of the atrium, however, did not alter in this plane.

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# PANCREATIC JUICE IS A RICH SOURCE OF THE ANTI-FATTY-LIVER FACTOR<sup>1</sup>

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Proof of the presence of an unidentified factor in pancreas that regulates fat metabolism rests on the observations that fatty livers develop in completely depancreatized dogs maintained with insulin and fed a high protein diet, and that this fatty liver can be prevented by the ingestion of as little as 60 mgm. per day of a choline-free fraction derived from raw pancreas (1). Since depancreatized dogs suffer from a reduction in plasma choline, it was suggested that depletion of choline stores accounts for the development of the fatty livers (2). Such an inference offers a reasonable explanation for 2 findings: a, the addition to the diet of free choline prevented the development of these fatty livers (3), and b, fractions of pancreas that contained the anti-fatty-liver factor were highly active in raising the level of plasma choline (2).

Since the addition of free methionine to the diet also prevented the development of fatty livers, it was concluded that the synthesis of choline from *free* methionine is not impaired in the completely depancreatized dog (4). It should be noted that this effect of methionine was found in dogs fed a diet rich in protein and hence not lacking in labile methyl groups. It was therefore suggested as a working hypothesis that the anti-fatty-liver factor of the pancreas is a proteolytic enzyme in the absence of which ingested proteins do not exert their full lipotropic effects (4).

According to the above interpretation, the anti-fatty-liver factor reacts with protein in the intestinal tract. This concept of the mechanism of action of the pancreas factor is supported by the observation that the ingestion of approximately 400 cc. of pancreatic juice per day prevented the onset of fatty livers in the depancreatized dog maintained with insulin (5). Dragstedt and his co-workers, however, have put forth the view that this anti-fatty-liver factor is an internal secretion of the pancreas and is not contained in the external secretion of this organ (6, 7). In view of these differences a further study of the lipotropic action of pancreatic juice was undertaken.

**EXPERIMENTAL.** Pancreatic juice was obtained from dogs by means of a modified Elman-McCaughan fistula (8). These juice-producing animals were maintained on a diet of lean meat and sucrose supplemented with vitamin concentrates and salts. They did not receive raw pancreas in their dietary mixture. Each animal also received intravenously 800–1000 cc. of Ringer's solution daily.

Nineteen normal dogs were depancreatized and maintained for the first few weeks thereafter on a lean meat-sucrose diet supplemented with raw pancreas as described in a previous paper (9). The raw pancreas was then omitted from the

<sup>1</sup> Aided by grants from the Christine Breon Fund for Medical Research.

dietary mixture and varying amounts of fresh pancreatic juice added to the diet as recorded in table 1. The feeding of pancreatic juice was continued for 16 to 22 weeks; at the end of this period the dogs were sacrificed and their livers sampled and analyzed after the manner previously described (10).

**RESULTS.** *Effect of feeding various amounts of pancreatic juice on liver fat.* An earlier study showed that the oral administration of 380-420 cc. of pancreatic juice per day prevented fatty livers. That pancreatic juice is highly active in this respect is clearly brought out by the results presented in table 1. Dogs

TABLE 1

*Effect of ingestion of pancreatic juice upon the liver lipids of depancreatized dogs maintained with insulin*

DOG NUMBER	WEIGHT		PANCREATIC JUICE		LIVER	
	When feeding of pancreatic juice begun	Final	Period fed	Amount per day	Weight	Total fatty acids
	kg.	kg.	weeks	cc.	gm.	per cent
D1		7.2	none	none	493	22.5*
D2		7.8	none	none	615	15.6*
D3		8.1	none	none	544	17.2*
D4	8.2	9.5	16	300	390	3.0
D5	7.3	8.5	16	300	382	3.1
D6	14.2	10.6	20	200	590	2.5
D7	14.5	12.4	20	200	722	1.7
D8	10.0	8.9	20	200	592	2.9
D9	8.8	9.1	20	100	624	2.6
D10	12.2	7.6	20	100	457	3.8
D11	7.9	9.0	20	100	432	2.4
D12	16.9	14.7	20	50	556	3.0
D13	13.2	11.7	20	50	644	2.3
D14	12.5	11.9	20	50	590	2.1
D15	7.2	8.5	20	20	360	4.2
D16	8.9	9.5	22	20	374	2.1
D17	8.8	8.4	20	20	294	2.9
D18	12.0	9.4	22	10	460	4.4
D19	10.5	5.6	22	10	260	1.9
D20	13.4	8.9	22	10	615	3.0

\* These dogs were sacrificed 20 weeks after pancreatectomy. During the last 18 weeks they did not receive either raw pancreas or pancreatic juice in their diets.

D1, D2 and D3 received no pancreatic juice; 20 weeks after pancreatectomy their livers contained from 16 to 23 per cent fatty acids. Dogs D18, D19 and D20 received 10 cc. of pancreatic juice daily for a period of over 5 months; their livers contained from 1.9 to 4.4 per cent fatty acids. The daily administration of this small amount of pancreatic juice (i.e., 5 cc. along with each meal) was just as effective in inhibiting the infiltration of fat in the liver as 200-300 cc. (table 1).

*Effect of pancreatic juice on plasma choline.* As pointed out above the principle in raw pancreas that prevents fatty livers in depancreatized dogs maintained with insulin is also concerned with maintenance of the level of plasma choline. The daily administration of various pancreas fractions that contained the anti-

fatty-liver factor not only prevented the fall of plasma choline in depancreatized dogs but raised the level in those in which the level of plasma choline had been permitted to fall (2). In order to compare the anti-fatty-liver factor of pancreatic juice with that of raw pancreas, the effect of pancreatic juice on plasma choline was studied.

Following pancreatectomy each of the dogs listed in table 2 was fed twice daily 250 grams of lean meat, 50 grams of sucrose, 10 grams of bone ash, and 1 gram of Cowgill's salt mixture (11). Each dog also received once daily 3 cc. of sardilene and 5 grams of yeast (9). Insulin was injected twice daily, imme-

TABLE 2

*Effect of feeding 50 cc. of fresh pancreatic juice per meal on plasma choline of depancreatized dogs maintained with insulin*

DOG NUMBER	DOG WEIGHT	TREATMENT	PLASMA CHOLINE
	<i>kgm.</i>		<i>mgm. per 100 cc.</i>
D21	10.2	Pancreatic juice feeding begun	29.2
	11.0	Pancreatic juice fed for 21 days	64.0
	9.9	Pancreatic juice not fed for 14 days	26.4
D22	6.7	Pancreatic juice feeding begun	33.4
	7.6	Pancreatic juice fed for 21 days	50.8
	6.9	Pancreatic juice not fed for 14 days	35.8
D23	5.8	Pancreatic juice feeding begun	47.8
	7.0	Pancreatic juice fed for 21 days	72.2
	6.1	Pancreatic juice not fed for 14 days	38.2
D24	6.7	Pancreatic juice feeding begun	40.6
	7.8	Pancreatic juice fed for 21 days	54.4
	7.0	Pancreatic juice not fed for 14 days	39.2
D25	9.8	Pancreatic juice feeding begun	22.6
	10.4	Pancreatic juice fed for 21 days	48.0
	10.0	Pancreatic juice not fed for 14 days	31.1

diately after the ingestion of the diet. Plasma choline was determined at various intervals after pancreatectomy by the method of Entenman et al. (12). When a drop below normal was definitely established, 50 cc. of pancreatic juice were added to each mixture fed for the next 21 days. Plasma choline values are recorded in table 2 for each dog on 3 separate occasions: 1, on the day that the feeding of pancreatic juice was begun; 2, on the last day (i.e., 21st day) of juice feeding, and 3, 2 weeks after the administration of pancreatic juice had been discontinued.

The administration of pancreatic juice for 3 weeks resulted in a significant rise in the concentration of plasma choline in all 5 dogs studied. In dog D21 the level of plasma choline was doubled by the addition of pancreatic juice to the diet. Two weeks after the ingestion of pancreatic juice had been discontinued, the levels of plasma choline fell to values that were in close agreement with those observed at the start of the experiment.



*Choline and methionine content of pancreatic juice.* Three samples of pancreatic juice obtained from as many dogs were analyzed for their content of total choline by the method of Glick (13) and methionine by the method of McCarthy and Sullivan (14). One hundred cubic centimeters of fresh pancreatic juice contained less than 1 mgm. of choline and less than 1 mgm. of methionine.

**DISCUSSION.** The results presented here confirm an earlier observation, namely, that orally administered pancreatic juice prevents the development of fatty livers in completely depancreatized dogs maintained with insulin (5). They show in addition, however, that the external secretion of the pancreas is highly active in this respect. The ingestion of as little as 10 cc. of pancreatic juice per day by a 10 kilo dog—a small fraction of the daily output by the pancreas—is sufficient to maintain a normal fat content in the liver.

The question arises as to the identity of the anti-fatty-liver factor contained in raw pancreas with that found in the external secretion of the pancreas. Two findings suggest that they are the same or entities that are closely related: 1, the anti-fatty-liver effects of pancreatic juice and of fractions prepared from raw pancreas cannot be accounted for by their choline or methionine content; 2, their ingestion increases the concentration of plasma choline in the completely depancreatized dog maintained with insulin.

#### SUMMARY

1. Pancreatic juice of the dog contains a factor highly active in the prevention of fatty livers of completely depancreatized dogs maintained with insulin. The daily ingestion of as little as 10 cc. (5 cc. added to each meal) was sufficient to keep the livers of such dogs normal for as long as 5 months.

2. Ingested pancreatic juice raises the concentration of plasma choline in the completely depancreatized dog maintained with insulin.

3. It is suggested that this factor in pancreatic juice is identical with that contained in raw pancreas.

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# INFLUENCE OF THE LIVER ON THE SHOCK PRODUCED BY EXTRACTS OF CERTAIN PARASITES

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The cystic fluid of *Echinococcus granulosus* produces severe to fatal shock when given intravenously to dogs. Recarte, Balea and Graña (1) and later Rocha e Silva and Graña (2) have shown that in some dogs there is an increased concentration of histamine in the blood accompanying the decreased blood pressure. However, the concentration of histamine was not proportional to the degree of hypotension.

The toxicity of extracts of certain parasitic worms when injected into dogs has been investigated by many workers (3). An extensive investigation was made by Shimamura and Fugii on extracts of *Ascaris* from swine. These authors isolated from this parasite an albumose-peptone which they designated as "ascaron." When this extract was given to normal dogs it produced severe shock, or more specifically, a marked decrease of arterial blood pressure. Rocha e Silva and Graña (4) studied the phenomenon and concluded that the liver is an important factor in the production of the hypotension. They described the response of the dog to injections of *Ascaris* extract as an anaphylaxis-like reaction because of its striking similarity to anaphylactic shock. Although as in anaphylactic shock the injection of *Ascaris* extract was followed by an increase of the blood histamine of the dog, histamine was not liberated from the perfused liver in response to *Ascaris* extract. Rocha e Silva and Graña (4) also found that the intravenous injection of hepatic glycogen in appropriate doses just before the injection of *Ascaris* extract reduced the degree of hypotension but did not affect the coagulability or histamine content of the blood.

The experiments reported in the present paper were designed to determine specifically the rôle played by the liver in the hypotension produced by injections of *Ascaris* extracts and hydatid cyst fluid. These substances were injected into normal adult dogs and pups, into hepatectomized dogs, into dogs with a fistula between the portal vein and the posterior vena cava and into dogs with a true Eck fistula with and without ligation of the hepatic artery. In other animals observations were made on the effect of injections of the extracts on pressure in the portal vein. Finally the effect of the extracts on the flow of blood from the perfused liver was observed.

**MATERIALS.** The hydatid cyst fluid used in these experiments was from cysts removed from sheep, calves and pigs. The cyst fluid from the different animals was pooled and preserved in sealed ampules. The *Ascaris suum* extract was prepared according to the method of Macheboeuf and Mandoul (5) by precipi-

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tating the triturated *Ascaris* material with 4 per cent trichloroacetic acid and dialyzing the filtrate for forty-eight hours to remove the acid. The extract was then neutralized before use and is designated as extract A. It apparently contains a glycogen-like material and a proteose-like substance. By adding alcohol to a final concentration of 50 per cent to this deproteinized extract the glycogen fraction was precipitated after twenty-four hours at room temperature. After neutralization the supernatant liquid was concentrated in vacuo in a water bath at 85°C. until it was reduced to a small volume and a precipitate was obtained by adding a great volume of acetone. The precipitate, which represents the toxic portion of the *Ascaris*, was collected and dried. It is designated as extract B. For injection into dogs extract B was dissolved in 10 ml. of isotonic saline solution and twice as much by weight of the glycogen fraction of extract A was added in an attempt to reconstitute the original substance.

In addition to these materials we also used a crude saline extract, which is designated as extract C. This material was prepared by adding an equal volume of physiologic saline solution to the volume of finely triturated *Ascaris suum*. After ten to fifteen minutes the material was centrifuged and the supernatant liquid was preserved by adding 1 cc. of 1:1,000 merthiolate to each liter. In all experiments the blood pressure was taken from a cannulated femoral or carotid artery.

Of the fifty dogs used in this investigation, forty-two were adults and eight were from two days to two months old. Observations were made on five animals on which a reverse Eck fistula had been performed and on nine on which total hepatectomy had been performed.

**METHODS AND RESULTS.** *Control observations.* In order to determine the proper dosage and to observe the desensitization or the rapid development of resistance or a refractory state (tachyphylaxis) following injections of *Ascaris* extract and hydatid fluid, observations were made on the blood pressure of a series of normal adult dogs anesthetized with pentobarbital sodium. The highly toxic effect of *Ascaris* extract is indicated by the fact that 0.1 ml. of extract A or 5 mgm. of extract B for each kilogram of body weight was enough to kill or to produce a profound shock in mature dogs (table 1). An intravenous injection of 0.1 ml. of extract C was frequently lethal. If the animal recovered, a second dose of the same size was without effect on the blood pressure (tachyphylaxis) but sometimes if a dose four times as great was given the response was as profound as that following the initial injection. Two milliliters of hydatid fluid for each kilogram of body weight produced a considerable decrease of blood pressure. After the blood pressure had returned to near the control level the same dose did not produce any alteration of the blood pressure (table 1). *Ascaris* extract did not desensitize the animal to the action of hydatid cyst fluid or vice versa. During the hypotension produced by these substances an inspection of the liver showed it to be markedly distended and very dark. Microscopic examination of specimens removed from the liver at this time showed severe congestion of the entire blood vascular system without hemorrhage. The parenchyma looked essentially normal.

TABLE 1

*Results obtained in normal dogs injected with Ascaris extracts and hydatid fluid*

DOG	WEIGHT, KGM.	MATERIAL INJECTED PER KGM.	BLOOD PRESSURE, MM. OF MERCURY		RESULT	TACHYPHYLACTIC EFFECTS
			Before injection	After injection		
1	6.8	1 ml. E.A.*	115	30	Death in 15 minutes	
2	8.1	0.5 ml. E.A.	130	30	Recovery in 3 hours	Reinjection of 2 ml. per kgm.: no effect
3	10.2	0.10 ml. E.A.	145	30	Death in 24 minutes	
4	6.2	0.10 ml. E.A.	140	50	Recovery	
5	9	0.20 ml. E.A.	160	30	Recovery	
6	25.5	0.10 ml. E.A.	150	30	Death	
7	10	0.10 ml. E.A.	130	20	Death	
8	12.5	0.10 ml. E.A.	160	30	Recovery	
9	10	0.05 ml. E.A.	150	80	Recovery	
10	17	0.10 ml. E.A.	120	20	Death	
11	14.6	0.10 ml. E.A.	140	20	Death	
12	15.4	0.10 ml. E.A.	150	12	Death	
13	8.4	5 mgm. E.B.*	170	20	Recovery	
14	8.5	5 mgm. E.B.	140	20	Death	
15	11.0	5 mgm. E.B.	130	10	Recovery	
16	14.0	1 mgm. E.B.	160	50	Recovery	
17	8.8	1 mgm. E.B.	180	40	Recovery	Reinjection of 2 mgm. per kgm.: no effect
18†	3.7	1 mgm. E.B.	180	40	Recovery	
19†	8.9	5 mgm. E.B.	150	40	Recovery	
20	8.5	2 mgm. E.B.	140	20	Recovery	

TABLE 1—*Concluded*

DOG	WEIGHT, KGM.	MATERIAL INJECTED PER KGM.	BLOOD PRESSURE, MM. OF MERCURY		RESULT	TACHYPHYLACTIC EFFECTS
			Before injection	After injection		
21	5.4	5 mgm. E.B.	140	10	Recovery	
22	4	10 mgm. E.B.	140	60	Recovery	
23	8.6	2 mgm. E.B.	130	25	Death	
24	14.8	1 mgm. E.B.	125	40	Death	
25	14	2 mgm. E.B.	170	80	Recovery	
26	6.5	0.10 ml. E.C.	190	30	Recovery	
27	9.5	0.10 ml. E.C.	140	30	Recovery	
28	27.5	0.20 ml. E.C.	100	40	Death	
29	12.5	0.10 ml. E.C.	130	70	Recovery	
30	16.7	0.10 ml. E.C.	160	20	Death	
31	8.1	2 ml. hydatid liquid	125	45	Recovery	Reinjection of 2 ml. per kgm.: no effect
32	9	2.5 ml. hydatid liquid	140	35	Recovery	
33	6.6	2.5 ml. hydatid liquid	120	70	Recovery	Reinjection of 2.5 ml. per kgm.: no effect

\* E.A. signifies injection of *Ascaris* extract A and E.B. signifies *Ascaris* extract B.

E.C. = crude extract of *Ascaris*.

† The injection of *Ascaris* extract in this dog was made in the splenic vein.

‡ This dog was injected before with 28 ml. of plasma of a liverless dog.

Extract A of *Ascaris* produced definite toxic manifestations when injected into dogs. In our experiments the glycogen fraction also appeared toxic for the dog, probably owing to the incomplete removal of the toxic fraction or extract B.

Injections of beta dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) have been shown to be capable of protecting guinea pigs against the bronchospasm produced by the proper dose of histamine (6). This drug has also been effective in reducing the severity of anaphylaxis in the guinea pig (7). Therefore we were interested in determining whether injections of benadryl would modify the action of injections of *Ascaris* extract. Premedication with intravenous injections of 6 mgm. of benadryl per kilogram of body weight did

not prevent the production of the typical hypotensive effect of injections of 0.1 ml. of *Ascaris* extract for each kilogram of body weight.

*Hepatectomized dogs.* After the liver had been removed by the three-stage method followed in this laboratory, anesthesia was continued with ether or pentobarbital sodium. In one instance local infiltration with procaine hydrochloride was used for cannulating the femoral artery after the animal had recovered from general anesthesia. When a dose of hydatid fluid or *Ascaris* extract B sufficient to produce deep shock or death in control animals was injected intravenously into hepatectomized dogs the effect on the blood pressure was negative or a slight transient decrease (10 mm. of mercury) resulted. Doses of 0.1 ml. per kilogram of body weight of the crude saline extract (extract C) or extract A caused rapid death in dogs having livers but liverless dogs were not killed by similar injections. The blood pressure of the hepatectomized animals decreased rapidly to shock level but invariably the blood pressure gradually increased later to a physiologic level. A second injection of the same dose or one four times as great was without effect (tachyphylaxis) (table 2). The series of liverless dogs used in these experiments afforded us an opportunity to observe the effect of trypsin on the blood pressure of such an animal. An intravenous injection of 2 mgm. of trypsin given to a hepatectomized dog caused a profound decrease of blood pressure and usually rapid death.

*Dogs with Eck fistulas.* From the results of the experiments on the liverless dog it seemed important to determine the response to these substances of dogs in which the hepatic circulation was altered in different ways. A series of observations was made on dogs with fistulas between the portal vein and the abdominal vena cava. The animals in this series responded to the injection of the *Ascaris* extract and hydatid fluid in the same manner as did normal dogs. In two dogs besides creating a true Eck fistula (the portal vein being ligated anterior to the fistula) the hepatic artery was ligated. There was only a very slow and moderate decrease of blood pressure following injections of *Ascaris* extract. In contrast to the results already described, an injection of hydatid cyst fluid in such dogs did not affect the blood pressure when given soon after the *Ascaris* extract.

*Transfusions.* Since the liverless dog was resistant to the material from the parasites, it seemed important to determine why. In order to see if the blood of a liverless dog contained a substance that prevented the shock, 28 ml. of plasma from a hepatectomized dog was given intravenously to a normal dog. Following the transfusion the dog responded to an injection of *Ascaris* extract by profound hypotension. The reverse of this experiment was done by taking 100 ml. of blood from a normal dog at the depth of *Ascaris* shock and injecting it into the liverless dog. The result was entirely negative. Blood taken from a liverless dog a short time after an injection of *Ascaris* extract caused profound hypotension when injected into a normal dog. These observations seemed to indicate that the liver plays a mechanical rather than a chemical rôle in the reaction of the dog to *Ascaris* extract and hydatid cyst fluid.

*Portal system pressures.* The marked congestion observed in the liver following injections of *Ascaris* extract and hydatid cyst fluid suggested that the pressure

TABLE 2

*Hepatectomized dogs injected with material from parasites*

DOG	WEIGHT, KGM.	MATERIAL INJECTED PER KGM.	BLOOD PRESSURE, MM. OF MERCURY		BLOOD PLATE- LETS, VOL. PER 100 CC.*		LEUKOCYTES PER CU. MM.		TACHYPHYLACTIC EFFECTS
			Before injec- tion	After injec- tion	Before injec- tion	After injec- tion	Before injection	After injection	
1	9.3	0.05 ml. 0.10 ml. E.A. 2.0 ml.	80 80 80	80 80 80	0.65	0.70			
2	13	0.10 ml. E.A.	120	80					Reinjection of 0.40 ml. per kgm.: no effect. Injection of 2 mgm. of tryp- sin per kgm.: deep shock
3	15	0.10 ml. E.A.	135	60					Reinjection 0.20 ml.: no effect. Injection of 2 mgm. of tryp- sin per kgm.: deep shock
4	17.5	0.10 ml. E.A.	115	40					Reinjection of 0.10 ml. per kgm.: no effect
5	10.2	5 mgm. E.B. 2.5 ml. hyda- tid liquid	100 100	90 100	0.25	0	2,250	2,050	
6	12	5 mgm. E.B. 2.5 ml. hyda- tid liquid	70 70	65 60	0.05	0.05	5,000	2,700	
7	13	5 mgm. E.B. 2.5 ml. hyda- tid liquid	120 120	120 120					
8	14	5 mgm. E.B.	110	110					Reinjection of 5 mgm. per kgm.: no effect. In- jection of 2 mgm. of tryp- sin per kgm.: shock
9	15.8	0.1 cc. E.C.	120	50					Reinjection of 0.20 ml. per kgm.: no effect

\* The measurement of platelets was made by use of the thrombocytoerit of Van Allen.

in the portal vein was increased. To observe the changes in the pressure of the portal vein the splenic or pancreatic vein was cannulated and connected to a water manometer. After an injection of *Ascaris* extract or hydatid fluid the pressure in the portal vein increased as the arterial pressure decreased. The pressure in the splenic vein in one experiment increased 69 mm. of water and in the pancreatic vein in one experiment it increased 200 mm. of water.

*The isolated liver.* Since a marked increase of the pressure of the portal system had resulted from injections of *Ascaris* extract, our interest was directed to the possible effect of the substance on the blood flow through the liver. To obtain information on this question a series of livers were perfused with defibrinated blood supplied to the cannulated portal vein from a reservoir placed at a height sufficient to give a perfusion pressure of 10 to 26 mm. of mercury to the blood. The blood was collected from the cannulated vena cava, the cannula having been placed in the vessel immediately above the diaphragm. The liver and blood were kept at an even temperature by appropriate heating devices. The flow from the liver was measured in graduated cylinders. When the flow had reached a steady state the *Ascaris* extract or hydatid fluid was injected into the tubing near the cannula in the portal vein. Almost at once the flow from the liver was greatly reduced and within two minutes it had declined from 85 to 35 per cent of the control outflow.

*Reactions of newborn pups.* It might be thought that the dogs used in these experiments had become sensitized to the material in the *Ascaris* extract through having been infected with other parasites of the dog (Brunner and his colleagues) (8). To test this hypothesis observations were made on four pups within forty-eight hours after birth. The blood pressure decreased to a very low level in response to an intravenous injection of 0.2 ml. of *Ascaris* extract for each 100 grams. of body weight. Newborn pups were relatively resistant to the crude extract (C), since they recovered very rapidly even from doses as large as 1 ml. for each 100 grams of body weight. The reaction of the pups to sufficient doses of *Ascaris* extract was in every way like that of the adult animals. Unless it may be shown that the pups were sensitized by materials that had passed the placental barrier it must be contended that the *Ascaris* extracts that we have used are primarily toxic for the dog, giving, on injection, an *anaphylaxis-like* reaction. In order to test whether infestation with a canine ascarid (*Toxocara canis*) increased the sensitivity of pups to *Ascaris suum* extract, a series of heavily infected pups two months old were observed. It was found in three experiments that rather than being sensitized they were highly immune to the *Ascaris suum* extract since the standard dose of *Ascaris* extract was without effect on the blood pressure of any of the heavily infected pups.

COMMENT. The decrease of blood pressure following the injection of the parasitic substances might reasonably be owing primarily to the retention of a large volume of blood in the hepatic portal system and secondarily to the liberation of histamine or some other toxic material from the liver. In a few experiments transfusions were given during the period of greatest hypotension with immediate and lasting beneficial effect, thus giving support to the idea that the hypotension was owing, at least in part, to an inadequate volume of circulating blood.



When the results of all of the experiments are taken into account, there is strong support for the belief that the purified *Ascaris* extract and the hydatid fluid act particularly on the hepatic venous system so that the resistance to outflow is markedly increased. The rise of portal venous pressure, the diminished outflow from the perfused liver and the extreme hepatic congestion are obviously the result of such a mechanism. The work of Deysach (9) on the "sphincter mechanism" of the liver of cats and rabbits and the experiments of Simonds (10) describe certain vascular arrangements and structures that possibly may be responsible for the shock produced by the parasitic materials. However, the fact that a mild reaction sometimes occurred in the liverless dog following the injection of purified extracts of *Ascaris* and profound hypotension often resulted from injections of the crude extract of *Ascaris* into hepatectomized dogs, indicates that the entire vascular effect cannot be charged to the liver alone. The mechanism responsible for the hypotension in the liverless animal after injections of extracts of parasites has not been determined.

The existence of a relative or partial desensitization and the increased coagulation time of the blood after the injection of *Ascaris* extract and hydatid fluid as well as the usual increase of histamine in the blood are strikingly similar to what is seen during anaphylactic shock in the dog. Manwaring and his associates (11) showed in their experiments that typical anaphylactic shock did not occur in the liverless dog. However, in a more recent publication Waters and Markowitz (12) reported that anaphylaxis occurred in a dog from which the liver had been removed in a single operation. The close similarity of the shock produced by injections of *Ascaris* extract and hydatid fluid to true anaphylaxis in the dog is clearly evident.

The question naturally arises as to whether we are, in fact, dealing with true anaphylaxis. A good case could be made for the affirmative but on close scrutiny the conviction grows that we are dealing with a physiologic response that possibly may be elicited by many agents. Our experiments show that desensitization is relative and not absolute. In the normal adult animal the initial injection of appropriate doses of *Ascaris* extract or hydatid fluid invariably caused profound hypotension. The fact that doses of the same size were without effect after recovery from the initial dose has been thought of as desensitization, but our experiments indicate that it is only relative or partial. In some dogs, doses four to ten times as great resulted in profound hypotension after recovery from the first dose, but the recovery was more rapid. This result was in sharp contrast to the complete desensitization usually observed in true anaphylaxis. The fact that newborn pups responded with a fall of blood pressure to injections of *Ascaris* extract indicates that previous infection with other parasitic material is unnecessary for sensitization. On the other hand three pups infected with *Toxocara canis* were immune to the intravenous injection of *Ascaris* extract A.

It is true that all of the evidence thus far presented points to the conclusion that the liver is the organ primarily involved in the shock resulting from injections of *Ascaris* extract or hydatid fluid. There is, however, one fact that should not be ignored; namely, that dogs that were ready for removal of the liver (pos-

terior vena cava and portal vein ligated anterior to the fistula) and dogs with true Eck fistulas and ligated hepatic arteries responded to injections of the parasitic material by a slow but moderate decrease of blood pressure which indicates that other organs and tissues besides the liver must participate in the reaction.

#### SUMMARY AND CONCLUSIONS

Extracts of *Ascaris suum* and hydatid fluid produce profound hypotension (shock) when injected intravenously in appropriate doses into dogs. Accompanying the decrease of arterial blood pressure there was a significant increase of the pressure of the portal system and grossly there was marked congestion of the liver and the intestines. The hepatic congestion was vascular since histologic examination of hepatic tissue showed the parenchyma to be free of hemorrhage. After recovery from a given injection of either *Ascaris* extract or hydatid fluid, reinjection of the same dose did not produce any change in the blood pressure (tachyphylaxis).

That the liver was chiefly responsible for the reaction has been shown by the fact that huge doses of hydatid fluid or purified *Ascaris* extract had little or no effect on the blood pressure in hepatectomized dogs. The crude extract of *Ascaris*, however, produced in liverless dogs a marked fall of blood pressure, from which all the animals recovered. After recovery they were refractory to the same or four times the original dose of the extract. Dogs whose hepatic circulation had been altered by an Eck fistula and ligation of the hepatic artery showed a small but very prolonged decrease of the arterial blood pressure. Dogs with Eck fistulas alone responded to the injections of the substances from the parasites in the same manner as did normal dogs. Newborn pups responded to intravenous injections of the purified extract with a markedly decreased blood pressure but pups heavily infected with *Toxocara canis* were immune to the extract.

The isolated perfused liver of the dog reacted to the injections of *Ascaris* extract by a marked decrease of hepatic outflow and very intense congestion.

Blood taken from a liverless dog after a large dose of *Ascaris* extract and injected into a normal sensitive dog produced the typical response of the blood pressure but when blood was taken from a normal dog at the depth of its hypotension from an injection of *Ascaris* extract and injected into a liverless dog it was without effect on the blood pressure, thus suggesting that the mechanism is primarily mechanical and only secondarily chemical.

From the evidence presented in this communication it may be concluded that congestion of the liver is the principal cause of the fall of blood pressure produced by *Ascaris* extract and hydatid fluid in susceptible dogs but other organs may also react as demonstrated by injection of the crude extract and deproteinized extracts of *Ascaris* into liverless dogs.

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# THE EFFECT OF EXPLOSIVE DECOMPRESSION ON CEREBROSPINAL FLUID PRESSURE<sup>1</sup>

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Changes in cerebrospinal fluid pressure as a result of exposure to decreased barometric pressures, independent of the effects of anoxia, have been reported by several authors. Armstrong (1939) observed a marked increase in the cisternal pressures of goats when these animals were decompressed. This increase was accompanied by the formation of visible gas bubbles in his manometer system. His published graph indicates that cerebrospinal fluid pressure began to rise at a simulated altitude of 18,000 to 20,000 feet and reached a value of 81.0 cm. H<sub>2</sub>O at 50,000 feet (87 mm. Hg). Armstrong also describes herniation of the brain through trephine holes in the skull, presumably the result of increased intracranial pressures, in animals subjected to low barometric pressures. These observations were confirmed by Walsh (1941) who described herniation of the scalp in a patient with a cranial defect, upon decompression to 28,000 feet simulated altitude (247 mm. Hg). Walsh and Boothby (1941) found an increase of about 30 mm. H<sub>2</sub>O in the spinal fluid pressure of men exposed to a pressure of 247 mm. Hg (28,000 ft.). They noted the appearance of bubbles in the manometer at 10,000 to 12,000 feet (523 mm. Hg to 483 mm. Hg). These bubbles disappeared at the final pressure.

On the other hand, Sutherland, Molumut and Brookes (1943) were unable to demonstrate significant rises in cerebrospinal fluid pressure even at pressures as low as 141 mm. Hg (40,000 ft.), but did observe bubble formation in their manometer system, and Peterson, Kent and Cone (1944) found no increase in intracranial pressure in a patient with a cranial defect at pressures as low as 226 mm. Hg (30,000 ft.).

In the course of studies on the physiological effects of explosive decompression, conducted in this laboratory during the past four years, experiments were carried out designed to determine the effect of this procedure on the cerebrospinal fluid pressure. The results of these experiments are reported in this paper.

**METHODS.** Preliminary experiments were performed along two lines suggested by the studies mentioned above. First, the skulls of a number of experimental animals were trephined and observations made to determine the occurrence and extent of herniation of the brain through the trephine holes during explosive decompression. Second, measurements of cerebrospinal fluid pressures were made by means of an open-tube manometer connected to a needle inserted into

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Ohio State University Research Foundation.

the cisterna magna. Following these preliminary observations it seemed desirable to follow cerebrospinal fluid pressure changes by means of a closed system. In this closed system the recording was done optically by means of a mirror mounted on a calibrated rubber membrane manometer. It must be kept in mind that cerebrospinal fluid pressure, like all physiological pressures, is always measured in relation to the pressure of the ambient atmosphere. This is necessary since the animal body is essentially a liquid system and atmospheric pressure is therefore transmitted instantaneously and without loss to its innermost parts.

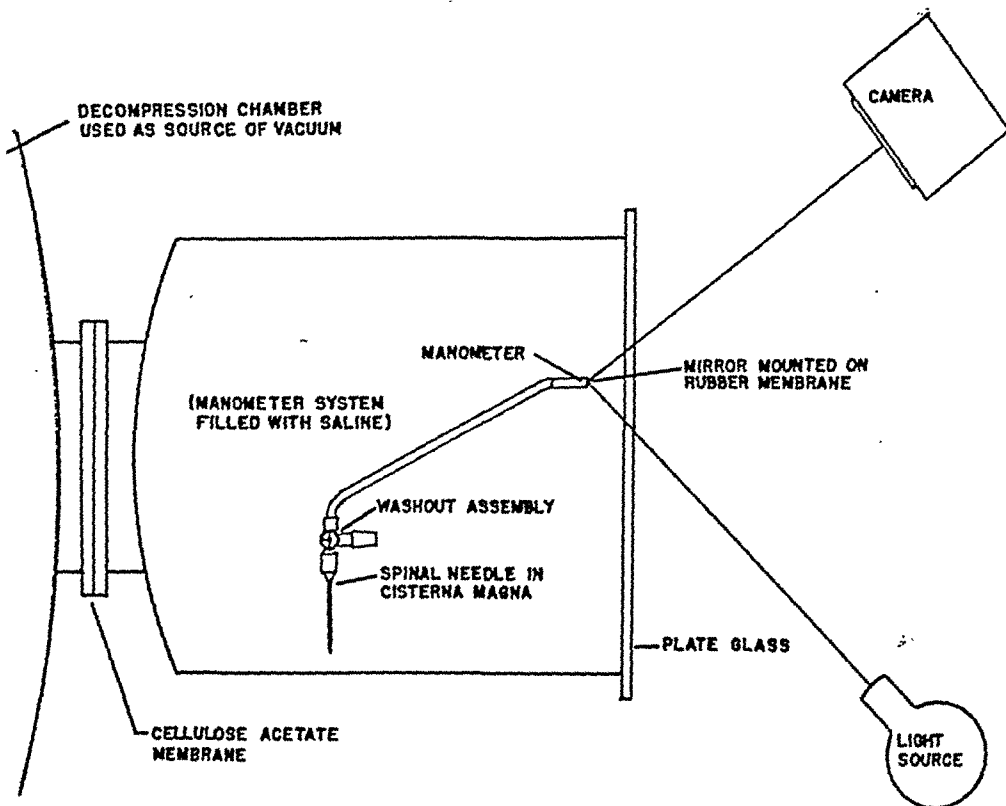


Fig. 1. Diagrammatic representation of the apparatus used in determining changes in cerebrospinal fluid pressure during explosive decompression.

It was therefore necessary in these experiments that the manometer be enclosed completely in the decompression chamber. The light source and the camera were outside the chamber, and the recording beam of light passed back and forth through a disc of  $\frac{3}{4}$  inch plate glass. The arrangement of the apparatus is shown in figure 1.

The optical manometer was connected by means of glass tubing and rubber pressure tubing joints to a 2-way metal valve to which a spinal needle could be attached. The system was filled with gas-free 0.9 per cent NaCl solution, scrupulous care being taken to remove all air bubbles from the system. The use of glass tubing greatly facilitated the detection of such air bubbles. Control decompressions of the manometer system, filled, but unattached to the animal, were carried out before each experiment. When the system was free of bubbles, the manometer would show no deflection when the ambient pressure was reduced.

The experimental animal (dogs were used, anesthetized with Nembutal, 30 mgm. per kilo, given intraperitoneally) was placed on his side on a dog-board, with his neck held in a flexed position. A spinal needle was then inserted into the cisterna, a few drops of fluid allowed to escape, and the needle connected to the 2-way valve of the manometer system which was turned so as to allow the spinal fluid to escape from the side arm. After the air in the valve had been displaced by cerebrospinal fluid, the valve was turned so as to connect the needle to the manometer and the experiment begun.

Explosive decompression was produced as previously described (Whitehorn, Lein and Edelmann, 1946). Rates and ranges used are given with the results. The animals were recompressed within one minute after the decompression. Oxygen was supplied by flooding the animal chamber at the moment of decompression.

**RESULTS.** In preliminary experiments on two rats, two rabbits and a guinea pig, all with trephined skulls, no brain herniations were observed when decompression from ground level to pressures as low as 54 mm. Hg (60,000 ft.) was carried out in 0.62 second (rate of pressure change 1124 mm. Hg per sec.). At final pressures lower than this, the rats and guinea pig showed some herniation, but the rabbits remained negative at final pressures as low as 34 mm. Hg (70,000 ft.).

One rabbit and one cat were observed with the usual type of open-tube manometer attached to the cisterna magna. These animals showed increases in cerebrospinal fluid pressure varying from about 1 mm. H<sub>2</sub>O with decompression from ground level pressure to 379 mm. Hg (18,000 ft.) to about 14 mm. H<sub>2</sub>O with decompression to 54 mm. Hg (60,000 ft.). The rate of decompression in all cases was 1124 mm. Hg per second. Bubbles usually appeared in the manometer in these experiments and the formation and escape of these bubbles through the open arm of the manometer made the pressure determinations with this system unreliable.

Satisfactory results with the closed manometer system were obtained in 25 experiments on 9 animals. The range of decompression in all cases was from 522 mm. Hg (10,000 ft.) to 141 mm. Hg (40,000 ft.). Two rates of decompression were used; in one the rate of pressure change was 4800 mm. Hg per second, the final pressure being reached in 0.08 second. In the other, the rate was 33,650 mm. Hg per second, the final pressure being reached in 0.01 second. Slow decompressions in which the final pressure was reached in six minutes were performed on three animals.

Decompressions at the explosive rates consistently produced a short-lasting rise in cerebrospinal fluid pressures. This rise was directly proportional to the rate of decompression. It averaged 11.6 mm. Hg in the case of the 4800 mm. Hg per second rate and 22.5 mm. Hg with the faster rate. A typical record is shown in figure 2. It can be seen that the pressure rises rapidly to its peak, and returns to the pre-decompression level in less than two seconds following the explosive decompression. The average duration of increased pressure was essentially the same with the two rates used. It averaged 1.51 seconds with the slower

and 1.56 seconds with the more rapid rate. In the slow decompressions which were carried out on three animals, there was a rise of cerebrospinal fluid pressure of about 3.0 mm. Hg (41 mm. H<sub>2</sub>O), which persisted as long as the animal was subjected to a reduced barometric pressure. In none of the experiments were bubbles observed in the manometer system. The results are summarized in table 1.

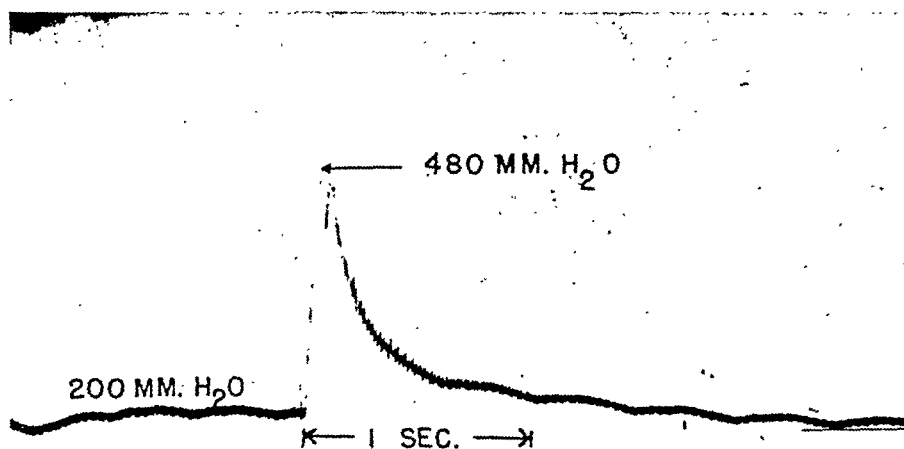


Fig. 2. Record showing the changes in cerebrospinal fluid pressure of a dog during explosive decompression from 522 mm. Hg to 141 mm. Hg (10,000 to 40,000 ft.) in 0.08 second. Average rate of pressure change 4,800 mm. Hg (93 p.s.i. per sec.).

TABLE 1

*The effect of decompression on cerebrospinal fluid pressure*

Range of decompression, 522 mm. Hg (10,000 ft.) to 141 mm. Hg (40,000 ft.)

RATE OF DECOMPRESSION	63.5 MM Hg PER MIN.	4800 MM. Hg PER SEC.	33,650 MM. Hg PER SEC.
No. of experiments.....	3	11	10
Average rise in c.s.f.p., mm. Hg.....	3.0	11.6	22.5
Average duration of rise in pressure, seconds.....	*	1.51	1.56

\* Pressure remained elevated until recompression.

DISCUSSION. A critical analysis of the results reported by Armstrong (1939) forces one to the conclusion that the large increase in cerebrospinal fluid pressure which he observed in his experiment on goats was due to the presence of gas bubbles in his manometer system. Such gas bubbles might have their origin in air set free from either the manometer fluid or from the cerebrospinal fluid after it had been forced into the manometer tube. Since the manometer system undoubtedly contained bubble nuclei, it is possible that air bubbles might have formed when the cerebrospinal fluid entered this system, even though no bubbles were formed in the spinal canal. Since bubbles were also seen in the manometer system by Walsh and Boothby (1941), it is possible that their results are not reli-

able although the values they reported are in line with those obtained by us with a closed system.

That our manometer system was gas free is shown by the fact that explosive decompression of the manometer system could be accomplished without any increase in the recorded pressure. Therefore the results reported in this paper could not have been affected by extraneous gas bubbles.

The outstanding feature of our results is a marked rise in cerebrospinal fluid pressure which occurs at the instant of explosive decompression and persists for a period of less than two seconds. The extremely brief duration of this elevation in the cerebrospinal fluid pressure (see fig. 2) is adequate proof that its cause is not the formation of gas bubbles. If such bubbles were formed, they would persist until the pressure was increased, and the gas forced back into solution. The cause of the increased pressure must, therefore, be sought in some other factor. The changes in intrathoracic and intra-abdominal pressures which occur during explosive decompression suggest themselves as possible causes. The brief duration of the rise in cerebrospinal fluid pressure and its relationship to the rate of decompression indicates that the causal factor is the change in intrathoracic pressure rather than the increased intra-abdominal pressure. The relationship between intrathoracic and cerebrospinal fluid pressures has been pointed out and discussed by Hamilton, Woodbury and Harper (1944). In order to test this relationship, a few experiments were run in which simultaneous determinations of cerebrospinal fluid and intrathoracic pressures were made using metal membrane manometers of the electrical capacitance type. While these studies are as yet incomplete, the records obtained indicate similarities in the shape, duration and magnitude of the curves which are in agreement with the relationships suggested by Hamilton, Woodbury and Harper (1944).

The results which we obtained with slow decompression in general agree with those of Walsh and Boothby (1941). The rises reported in this paper are so small as to be of doubtful significance. Indeed, Sutherland, Molumut and Brookes (1943) consider rises of this magnitude within the limits of normal variation. But if we concede that these changes are significant, then it is not impossible that they might be the result of bubble formation although a persistent rise in intra-abdominal pressure might with equal validity be considered the causal factor.

While we believe that our experiments offer convincing evidence against the theory that bubbles can be produced explosively by a sudden drop in barometric pressure, we must nevertheless recognize the possibility that bubbles may be formed if the animal is maintained at reduced pressures for a sufficient time. If a time factor is necessary for the formation of such bubbles, we would have in this fact an explanation of the persistent but small rise in cerebrospinal fluid pressure which occur with slow decompression. The cases of herniation of the brain which sometimes occurs when trephined animals or human beings with cranial defects are subjected to extreme reduction of the barometric pressure might also be explained on this basis. If a time factor is necessary for bubble formation, this would also explain why no such persistent rise occurs when the decompression is explosive in nature. Under these conditions, the animal is not maintained at



the reduced barometric pressure for a sufficient time for the formation of bubbles to occur.

The marked but brief rise in cerebrospinal fluid pressure which follows explosive decompression may bear a causal relationship to the hemorrhages occasionally seen in the walls of the lateral ventricles.

#### SUMMARY

The effect of both slow and explosive decompression on cerebrospinal fluid pressures of anesthetized dogs has been measured by means of a closed optical manometer system.

Decompressions to 141 mm. Hg (40,000 ft.) in about 10 minutes resulted in an average rise of 3.0 mm. Hg which persisted as long as the reduced pressure was maintained. Explosive decompression from a pressure of 522 mm. Hg (10,000 ft.) to one of 141 mm. Hg (40,000 ft.) in 0.01 or 0.08 second resulted in pressure rises of 22.5 mm. Hg and 11.6 mm. Hg respectively which persisted for only 1.5 seconds. No bubbles or evidences of bubble formation were observed in the course of these experiments. The rise in cerebrospinal fluid pressures is considered to be a reflection of a similar rise in the intrathoracic pressure.

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## MECHANISMS OF ANTIDIURESIS IN THE DOG AND IN THE RAT

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In 1937 Gilman and Goodman (1) found that in NaCl diuresis or by withholding water from rats considerable amounts of the antidiuretic hormone are excreted in the urine. In agreement with Verney's suggestion (2) they advanced the general theory (3) that dehydration stimulates the posterior part of the pituitary gland and thus evokes a physiological counterregulation against the loss of body water. The central rôle of the pituitary in antidiuresis was again emphasized in recent years by the work of M. Pickford and de Bodo. Pickford (4) found that acetylcholine produces antidiuresis in atropinized dogs regardless of whether their kidneys are innervated or denervated, but not in hypophysectomized animals. Following this lead, de Bodo and co-workers (5) investigated morphine and phenobarbital antidiuresis and came to the result that the drugs stimulate the secretion of the antidiuretic hormone. They based this conclusion mainly on the fact that in "neurohypophysectomized" dogs the drugs are no more antidiuretically active. All these findings seem to suggest that the old conception of Molitor and Pick (6) of a water regulating center which is depressed by opiates and certain other central anesthetics will have to be revised (see Pickford (7)).

We tried to extend first the information given by Gilman and Goodman on the stimulation of the hypophysis by dehydrating agents, and used one of the potent new diuretics, melamine, which was recently described (8), and caffeine, comparing them with NaCl in rats.

**METHODS.** Like Walker (9) and Schaffer, Cadden and Stander (10) we were not able to duplicate the procedure described by Gilman and Goodman which was based on the possibility of removing urea and salts from a solution of the antidiuretic hormone by dialysis through cellophane membranes of 0.00072 inch thickness. In many experiments with pitressin-saline mixtures containing 10 to 50 m.u./cc. and 0.25 per cent acetic acid which were dialyzed in such cellophane bags against running tap water, three hours were found necessary to remove the crystalloids almost quantitatively, in agreement with the authors. However, at the same time the liquids had lost 40 to 80 per cent of the antidiuretic principle into the dialysates. The same was observed in experiments in which such mixtures containing in addition caffeine were dialyzed.

Therefore the method was modified after it had been found that a sodium chloride solution of up to 10 per cent containing 5 to 10 m.u./cc. pitressin can be assayed satisfactorily with the method of Burn (11), provided that not more than 2 cc./kgm. are injected into well hydrated rats.

It became necessary to remove the diuretics or drugs other than sodium chloride by specific procedures before concentrating and assaying the urine. In the case of caffeine or theophylline, this was done by chloroform extraction of the urine acidified with acetic acid in a Soxhlet apparatus for 6 to 7 hours. Morphine was removed from urine by alkalizing it with ammonia in the cold and extracting five times with chloroform. Phenobarbital containing urine was acidified with acetic acid, and the drug extracted at room temperature with ether. In the case of melamine or formoguanamine, the drug was precipitated by powdered oxalic acid of a final concentration of 1 per cent, and centrifuged. The excess of oxalic acid was removed by a slight excess of powdered  $\text{CaCO}_3$ , and the centrifuged clear liquid was admixed with concentrated acetic acid to a final concentration of 0.25 per cent. That these procedures do not interfere with the antidiuretic hormone was ascertained in a series of experiments in which pitressin was added to urines containing the drugs.

Normal urine which had been in contact with feces for a short time, then was filtered clear and admixed with pitressin, lost rapidly its antidiuretic activity even when cooled in ice. This shows that destruction of the antidiuretic principle by solutes rather than its mechanical adsorption to stool particles as Gilman and Goodman assumed caused the loss in activity. There are reasons to believe that the process in question is a hydrolysis by bacterial enzymes. This point will be investigated. At any rate, it is not sufficient to separate the urine from stool by mesh or filtration.

The following standard procedure therefore was adopted: As much of the urine as possible during diuresis was collected by holding the rats individually and repeatedly over a funnel with filter paper on a graduated cylinder which stood on the screen covering the top of the metabolism cage (12), so that feces remained outside the funnel and the urine used for the assay was not contaminated. A second part of the urine was collected in the usual way, and the total volume of urine was used for calculating the amount of antidiuretic hormone excreted. After the urine was freed from the administered drug, as described above, it was acidified with acetic acid, concentrated in high vacuum at less than  $30^\circ\text{C}$ . bath temperature and brought to a definite volume with distilled water keeping the fluid always acid. Burn's method was used for the assay.

**EXPERIMENTAL.** 1. *Does Dehydration of Rats by Diuretics Stimulate the Secretion of the Antidiuretic Hormone?* *NaCl diuresis.* In three experiments forty rats deprived of food and water for 16 hours were fed 50 cc./kgm. fluid. Twenty-four animals received 5 per cent NaCl solution, the remaining control animals saline. The rats in NaCl diuresis excreted in 7 hours 10.0, 11.5 and 15.9 m.u./kgm./hr., respectively, of antidiuretic principle. When, in the last experiment, the concentrated urine containing 8 m.u./cc. hormone was brought to 1 per cent HCl and refluxed for 30 min. at  $100^\circ\text{C}$ . it had lost all antidiuretic

activity; when, however, it was heated only to 90–93°C. the inactivation was incomplete. Both observations correspond to those made with pitressin solutions of similar strength.

*Melamine diuresis.* By the same procedure, the twenty-four rats (which eleven days later excreted in NaCl diuresis 15.9 m.u./kgm./hr. hormone) were fed 500 mgm./kgm. melamine hydrochloride in 25 cc./kgm. saline. They excreted 189 per cent, the saline control animals 20.1 per cent of the fluid fed in 7 hours. In the 5 times concentrated melamine urine no antidiuretic hormone was found. In a similar experiment the melamine rats excreted in 6½ hours 203.7 per cent of the fluid fed. This urine 7.5 times concentrated was free from antidiuretic hormone. On the following day, the same rats, without having received food or water, were again fed melamine and excreted in 7 hours 137 per cent of the fluid fed. In the 3.5 times concentrated urine 5.7 m.u./kgm./hr. antidiuretic hormone were found to be excreted. None was found in three more experiments in which a strong diuresis was produced by 200 mgm./kgm. melamine or 500 mgm. of the HCl salt.

*Caffeine diuresis.* In two experiments rats were fed 75 mgm. and 150 mgm./kgm. caffeine respectively. The urine was collected for 6 hours and, after removal of the drug, assayed; no appreciable amount of antidiuretic hormone was found.

From these and one of the following experiments it appears that diuresis only if produced by hypertonic NaCl solution, but not by water, melamine or caffeine, is accompanied by excretion of the antidiuretic hormone. Since it is known that melamine as well as caffeine removes not only large amounts of water but also of salt (8) the effect of NaCl administration or of thirst has to be considered as a salt effect (electrolyte imbalance (13)) rather than a dehydration effect upon the pituitary gland. In this respect the result of the repeated melamine administration to the same rats on two successive days is remarkable: the dehydration by melamine as such which is accompanied by a considerable loss of NaCl was ineffective in provoking hormonuria whereas, in the course of the second melamine diuresis, the thirsting animals lacking now more water than salt excreted moderate amounts of the antidiuretic hormone.

2. *The Antidiuretic Action of Morphine, Acetylcholine and Phenobarbital on Dogs.* As a next step, de Bodo's findings in dogs were reinvestigated. His results concerning the antidiuretic action of morphine or phenobarbital in water diuresis were duplicated. It was found that not only the urinary excretion was decreased but also that the concentration in coloring matter rose subsequent to the morphine injection. This parallels the trend of the concentration of urine chlorides the rise of which was followed by de Bodo. Two new facts were found in the course of this investigation which appear to corroborate de Bodo's theory. In a dog with denervated kidneys the same marked antidiuresis as in normal dogs was produced by morphine. In either case pitressin, too, was strongly active. Furthermore it was found that dogs in water diuresis when injected with morphine excrete considerable amounts of the antidiuretic hormone matching in magnitude those excreted by rats in NaCl diuresis or when thirsting as reported

by Gilman and Goodman and in this paper. On three dogs weighing between 5.5 and 7 kgm. seven morphine experiments were performed following de Bodo's procedure. In dog no. 5 the kidneys of which had been denervated one to ten weeks before the experiments, the urine of the pre-periods showed no significant amount of antidiuretic hormone. During the morphine antidiuresis 20.5, 0, 9.1 m.u./kgm./hr. were excreted. The two other dogs, nos. 16 and 30, with intact kidneys excreted under morphine 27.8, 15.6, 10.5, 14.4 m.u./kgm./hr. of antidiuretic hormone which was inactivated by hydrolysis with 1 per cent HCl. In one instance, 1.7 m.u./kgm./hr. were found to be excreted in the pre-period.

However, in contrast to de Bodo's findings, a remarkable difference appeared between the activity of pitressin and morphine when urinary flow was produced by diuretics such as formoguanamine (8). Whereas pitressin was inactive in this instance in small or large doses, in agreement with the results of previous workers and with our own work on rats, morphine proved to be as strongly antidiuretic in formoguanamine diuresis as in water diuresis. This was found the case in normal dogs as well as in animals with denervated kidneys.

Examples: 1. The mean urinary excretion of a normal female dog, fasted for 16 hours, was first established in a pre-period of three hours. After 40 mgm./kgm. of formoguanamine were fed the dog excreted 0.92 cc./5 min. over normal between the 1st and the 3rd hour. Then 5 mgm./kgm. morphine sulf. were injected subcutaneously. In the subsequent hour the urinary excretion dropped to 0.12 cc./5 min. and, in the fifth hour, was increased again to 0.63 cc./5 min. over normal. 2. Similarly, the urinary excretion per 5 minutes of a dog with denervated kidneys was during the first 60 minutes after formoguanamine 0.04 cc., the next 60 minutes 0.76 cc., and the following 30 minutes 1.2 cc. over normal. In the three hour period after morphine injection the corresponding excretion rates were 0.08, 0.02, 0.1 cc.

The urine excreted during formoguanamine diuresis contained no antidiuretic hormone. However, after morphine injection 5.3, 3.6, 2.2 m.u./kgm./hr. of the hormone were excreted. The phenomenon will be discussed later on when the corresponding experiments on rats with various diuretics have been reported.

Under the same conditions, acetylcholine in atropinized dogs proved strongly antidiuretic if tested in water diuresis, but it failed to counteract formoguanamine diuresis.

Example: A normal animal in water diuresis excreted 20 cc./5 min. during the last 40 minutes before, and 5.2 cc./5 min. during 60 minutes following the intravenous injection of 5 mgm. acetylcholine iodide. However, in formoguanamine diuresis, the urinary excretion over normal was 2.35 cc. before acetylcholine, and 2.4 cc./5 min. after the injection.

In both respects acetylcholine behaves in a manner similar to that of the antidiuretic hormone of which Pickford suggests that its production is stimulated by the release of acetylcholine.

*Sodium phenobarbital* was markedly antidiuretic in water diuresis when 50 to 80 mgm./kgm. were injected intravenously into dogs with innervated—dogs nos.

16 and 69—or denervated—no. 5—kidneys, independently of whether the animals appeared excited or depressed by the drug. However, in all four experiments the urine concentrated 4 to 9 times failed to show any antidiuretic activity, when assayed on rats. In formoguanamine diuresis, phenobarbital was antidiuretically inactive and resembles in this respect acetylcholine or the antidiuretic hormone.

3. *The Antidiuretic Action of Morphine and Other Central Anesthetics on Rats.*

a. *Rats with innervated kidneys. Water diuresis.* Similar precautions were used with rats as with dogs in order to ascertain that antidiuresis by morphine and other drugs was genuine. Interference of the drug with the normal absorption rate of water must be excluded. There remained to make sure that retention of urine in the bladder under the influence of the drug will not create a source of error. Therefore in each experiment three groups of 8 rats each were fed 50 cc./kgm. of tap water. After exactly one hour the bladders were emptied by pulling the tail of each animal, and the volumes of the excreted urine were recorded. Two cc./kgm. of saline containing various doses of the drug were injected subcutaneously. Exactly one or two hours after injection the animals were killed by a high concentration of chloroform and the excised bladders were drained. The results of representative experiments with antidiuretically active central anesthetics are shown in the following figures indicating the urinary excretion in percent of fluid administered, during the period of drug action; the doses in mgm./kgm. are given in parenthesis. Morphine sulf.: (0), 62.3; (10), 23.4; (20), 11.9. Codeine phosph.: (0), 68.4; (50), 39.6; (100), 35.5. Dicodid HCl: (0), 40.8; (2.5), 22.7; (5), 16.3. Sodium phenobarbital: (0), 51.8; (25), 34.6; (50), 22.2. Nembutal: (0), 50.6; (25), 38.1; (50), 25.9. Demerol: (0), 44.6; (25), 25.2; (50), 17.7. Neither phenobarbital nor nembutal in the doses given produced a retention of urine in the bladders due to ureteral spasm, in contrast to the other substances.

Several similar experiments were done with dl-1.2-diphenyl-2-aminoethanol hydrochloride and the corresponding iso-compound which had been described by Dodds and co-workers (14) as substitutes for morphine. However, two years ago, in preliminary experiments on dogs which were injected subcutaneously with 10 and 100 mgm./kgm. respectively of these compounds, we were unable to detect any central depressant action, the only effect of the higher dose being vomiting. In rats, doses of 100 mgm./kgm. and more produced marked excitation and diuresis, and no antidiuretic component in their actions was apparent. It is interesting in this connection that Dodds, Lawson, Simpson and Williams in a recent publication (15) came to the result that in contrast to morphine and pethidine (demerol) their compounds do show no increase in pain threshold in rats and that "only the particular pain associated with pressure on nerve caused by malignant growths seems to be affected by the new compounds. . . in man".

*Diuresis produced by diuretics.* Both in rats and in dogs pitressin and morphine differ in their action upon urinary excretion when stimulated by diuretics such as sodium chloride, melamine or formoguanamine. In all instances 50

to 500 m.u./kgm. pitressin were not significantly antidiuretic in rats; 20 mgm./kgm. morphine injected subcutaneously ninety minutes after the diuretic was given produced a very marked antidiuresis quite similar to that produced in water diuresis. In representative experiments the urinary excretion, in percent of fluid administered, during 2 to 3 hours after morphine injection was the following; the corresponding figures for the control rats are given in parenthesis. Diuresis produced by 50 cc./kgm. of 5 per cent NaCl: 29.0 (58.6); by 25 cc./kgm. of saline with 125 mgm./kgm. melamine: 12.3 (61.7); 200 mgm./kgm. melamine: 33.8 (66.1); 10 mgm./kgm. formoguanamine: 10.5 (50.3).

On the other hand, sodium phenobarbital injected subcutaneously when diuresis was produced by 2.5 grams/kgm. NaCl or by melamine or formoguanamine did not act antidiuretically but, in proportion to the dose, even somewhat increased the urinary excretion.

*Excretion of the antidiuretic hormone during morphine antidiuresis.* The first evidence that water output is counteracted by morphine in the rat mainly by a mechanism different from that in the dog was obtained when the amount of the antidiuretic hormone excreted by hydrated rats under morphine was determined. In two experiments thirty-two rats weighing about 5.5 kgm. were fed 50 cc./kgm. of tap water and injected subcutaneously with 20 mgm./kgm. morphine sulfate in 2 cc./kgm. saline. Eight control rats were treated in the same way but were injected only with saline. The morphine antidiuresis was very marked. Two and two-tenths and 2.9 m.u./kgm./hr., respectively of hormone were found to be excreted in 6 hours. In a third experiment, 32 rats which had been deprived of water for eighteen hours received 500 mgm./kgm. melamine HCl in 25 cc./kgm. saline orally and 20 mgm./kgm. morphine sulf. in 2 cc./kgm. saline subcutaneously. The antidiuresis of the morphine rats as compared with the control rats was marked. Only traces of antidiuretic activity were found in the urine.

b. *Rats with denervated kidneys. Technique.* Altogether, 134 male rats weighing 140 to 290 grams were operated on. They were anesthetized with ether. Skin incision at the right side was made parallel to and about 0.5 cm. below the last rib, and the abdominal muscles were separated; the intestines and the liver were pushed away from the kidney by cotton pads saturated with warm saline. The kidney was stripped with fine forceps of both fatty and fibrous capsule starting at the lower pole, working around the upper lobe, and ending about 1 cm. beyond the hilus. This was carefully freed from connecting and nervous tissue on all sides, complemented by rubbing of the vessels and the ureter with small cotton plugs moistened with 10 per cent FeCl<sub>3</sub> solution and then with saline. Rupture of kidney vessels was avoided. The organs were replaced, the wound closed, and the same operation was performed on the left side; here the cut was placed about 1 cm. below the last rib. Care was taken not to bring other organs in contact with the FeCl<sub>3</sub>. The duration of anesthesia did not exceed 45 minutes. After two days to one month the animals were used for repeated experiments, often in crossed tests.

Rats with denervated kidneys produced in most instances urine of lighter color

than normal ones, and the reaction of the urine to litmus paper was, with few exceptions, amphoteric or alkaline rather than acid. It also appeared (14 expts.) that the urinary excretion in five hours after oral administration of 25 cc./kgm. saline was on the average almost double ( $44.2$  per cent  $\pm 3.29$ ) that of normal rats ( $24.0$  per cent  $\pm 1.52$ ) (12). On the other hand, the excretion after 50 cc./kgm. of tap water was lessened, e.g., in one representative experiment comprising two groups of 8 rats each, 57.8 per cent, the corresponding excretion in normal rats being 93.9 per cent. In principle, however, tap water as well as

TABLE 1  
*Experiments on rats*

FED 25 CC./KGM. SALINE CONTAINING  mgm./kgm.	INNERVATED KIDNEYS		DENERVATED KIDNEYS	
	Urinary excretion in 5 hrs. in per cent of fluid fed	Increase over controls (= 100) by per cent	Excretion in 5 hrs. in per cent of fluid fed	Increase over controls (= 100) by per cent
Urea..... 2000	119.8	356	109.2	456
Melamine..... 200	101.7	227	97.7	166
Adenine sulf..... 50	103.7	295	96.3	148
	74.7	304	114.9	74
Formoguanamine..... 10	115.5	472	61.0	11
	84.7	443	66.3	31
	90.3	209	66.0	56
	91.4	365	107.2	154
	88.9	163	87.6	63
Caffeine..... 37.5	60.5	253	42.6	77
	48.3	138	67.5	23
	40.1	58	55.3	17
	37.4	24	58.3	80
FED 50 CC./KGM. WATER AND INJECTED SUBCUTAN. MU./KGM. PITRESSIN	Time to maximum rate of excretion in minutes			
0	102	111	100	101
20	189	183	199	191
				96
				144

urea, melamine, adenine, formoguanamine or caffeine produced a significant diuresis in operated rats in the same dose range as in normal animals. The same small doses of pitressin also were antidiuretic in water diuresis independently of whether the kidneys were innervated or denervated, and ineffective in diuresis produced by the aforementioned diuretic substances (table 1).

In contrast to these findings and to the results in the dog with denervated kidneys, morphine produced only a very slight antidiuretic action in rats with denervated kidneys when subjected to water diuresis and no antidiuretic action at all in melamine diuresis. It should be emphasized that the other typical



effects of morphine were present in these animals: they were limp and showed hardly any pain reaction; the eyes were bulging and anesthetic. Micturition was inhibited by the spasm of the bladder sphincter muscle, so that the bladders were full to bursting after two hours. The particular mechanism of morphine antidiuresis in rats became still more conspicuous when it was found that the antidiuresis by sodium phenobarbital was not diminished after denervation of the kidneys. The figures for the urinary excretion during 1 to 3 hours after subcutaneous injection of the anesthetics will illustrate this when they are compared with those in parenthesis, of the similar control rats.

	KIDNEYS DENERVATED	KIDNEYS NORMAL
a. 20 mgm./kgm. morphine sulf. water diuresis.....	29.7 (44.7), 29.7 (36.1)	10.7 (38.2), 21.0 (45.6)
melamine diuresis.....	64.2 (62.5), 83.9 (67.4)	33.8 (66.1), 32.8 (46.9)
b. 100 mgm./kgm. sodium pheno- barbital water diuresis.....	32.8 (52.6)	47.8 (70.1)

DISCUSSION. The mechanism of antidiuretic effects is not a single one. It is of special interest to note that the main mechanism of antidiuretic action of morphine differs in different species of animals, as dog and rat. In dogs as well as in rats, the antidiuretic hormone from the posterior lobe of the hypophysis plays a rôle in producing antidiuresis. It is secreted under the influence of an increased sodium chloride concentration—not of dehydration—and more specifically by acetylcholine or impulses which lead to release of acetylcholine. In the dog all this proved to be independent of the presence of renal innervation. The antidiuretic hormone does not counteract diuresis produced by diuretics, nor does, therefore, acetylcholine.

Phenobarbital appears to act via this mechanism in suppressing central impulses by which either acetylcholine action or directly the secretion of the antidiuretic hormone is inhibited, for the following reasons: 1, it is antidiuretically active in dogs or rats with innervated or denervated kidneys; 2, is not active in "neurohypophysectomized" dogs, and 3, is inactive in diuresis produced by diuretics either in dogs or in rats. The only unsatisfactory point in this conception of phenobarbital action is the fact that no antidiuretic hormone was found in the urine of dogs treated with antidiuretic doses of phenobarbital. But since it is known that the antidiuretic hormone is excreted in the urine only when a high blood level is reached it may be argued that the secretion of the hormone is not stimulated strongly enough by the drug as to reach the critical blood level. It seems consistent with this interpretation that the antidiuresis by phenobarbital is, though marked, less outspoken than that produced by morphine.

*Morphine* itself reveals three quite different mechanisms of antidiuresis. In the *rat* the main pathway to this effect is the renal nerves. Morphine antidiuresis during the action of diuretics, and most of it in water diuresis, is paralyzed when the kidneys are decapsulated and denervated. Furthermore, only

small amounts of the antidiuretic hormone were found in the urine of normal hydrated rats under morphine. This agrees with the recent observation of O. S. Gibbs and Fulghum (16) that morphine—in contrast to pitressin—does not increase the output of chlorides in rats, from which observation they concluded that “morphine probably does not act via the pituitary mechanism in the rat”. In the *dog*, the nerves of the kidney seem to play no rôle in morphine antidiuresis. In water experiments, morphine produced antidiuresis regardless of whether the kidneys were innervated or denervated; and rather large amounts of the antidiuretic hormone were excreted in the urine. Since de Bodo found that under these conditions the chloride output is increased and that in “neurohypophysectomized” dogs morphine does not act antidiuretically, the pituitary mechanism of the water antidiuresis of morphine is well established. The assumption of a third mechanism of morphine antidiuresis becomes necessary because in diuretic diuresis, produced by sodium chloride, melamine or formoguanamine, morphine is antidiuretic, i.e., it is effective under conditions where acetylcholine, pitressin or phenobarbital are inactive. The mechanism of this effect is still under investigation. There are several possibilities: 1, a peripheral action upon the kidney; 2, a depressing effect upon the adrenocortex which is active in controlling the salt equilibrium in the body; 3, a depressing action upon the anterior lobe of the hypophysis which in turn normally stimulates the adrenocortex and is essential for diuresis (17). Since denervation of the kidneys did not influence the morphine antidiuresis even after many weeks and, on the other hand, “neurohypophysectomized” dogs did not show antidiuretic morphine activity, the third possibility seems more likely as a working hypothesis at the present time.

The facts and arguments here presented justify M. Pickford's cautious view (7) on the “control of the secretion of antidiuretic hormone from the pars nervosa of the pituitary gland”, in which she leaves open the possibility of other antidiuretic factors.

The results presented here are not at variance with the conception that the immediate site of attack of antidiuretic substances like morphine or phenobarbital is located in the central nervous system. By the central anesthetics impulses are suppressed which normally travel to all parts of the hypophysis (18), the adrenocortex or the thyroid, and participate in the regulation of body water.

#### SUMMARY

1. Rats fed 2.5 grams/kgm. NaCl excrete 10 to 16 m.u./kgm./hr. of antidiuretic hormone. In the urine of rats which received other dehydrating substances like melamine or caffeine only insignificant amounts of antidiuretic hormone were found.

2. Dogs with innervated or denervated kidneys during water diuresis excrete 9 to 28 m.u./kgm./hr. of the antidiuretic hormone under morphine which is antidiuretically active. In spite of the fact that neither the antidiuretic hormone nor acetylcholine counteract diuresis produced by the diuretic formoguanamine, morphine is antidiuretic also in this instance; then 2 to 5 m.u./kgm./hr.

of the antidiuretic hormone are excreted. This points to a humoral mechanism other than that originating in the posterior lobe of the pituitary gland.

3. Phenobarbital is antidiuretically active in such dogs during water diuresis but not during diuretic (formoguanamine) diuresis. No antidiuretic hormone was found excreted during phenobarbital antidiuresis.

4. Water diuresis in rats with innervated kidneys is counteracted by morphine, codeine, dicodid, sodium phenobarbital, nembutal or demerol. dl-1,2-diphenyl-2-aminoethanol is not antidiuretically active. Only small amounts of the antidiuretic hormone, from traces to 3 m.u./kgm./hr., were found in the urine during morphine antidiuresis.

5. Diuretic diuresis produced in such rats by NaCl, melamine, or formoguanamine, is not antagonized by pitressin or phenobarbital but is counteracted by morphine.

6. Rats with decapsulated and denervated kidneys respond to water, urea, melamine, adenine, formoguanamine or caffeine with diuresis. Pitressin or phenobarbital are antidiuretically active during water diuresis, but the main antidiuretic effect of morphine is eliminated by the operation, and no antidiuretic effect at all remains during diuretic diuresis produced by melamine.

7. Three different mechanisms of antidiuresis are apparent in these experiments: 1, the acetylcholine-antidiuretic hormone (posterior lobe of the pituitary) mechanism; 2, nervous control of the kidney of the rat; 3, a humoral factor in the dog different from the antidiuretic hormone.

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# THE EFFECTS OF MORPHINE ON DOGS IN HEMORRHAGIC AND TRAUMATIC SHOCK<sup>1</sup>

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Numerous studies have been made on the physiological effects of morphine on normal dogs (1, 2, 3), but so far little definitive work has been done on the effects of this drug in shock. Nevertheless, morphine has been employed in cases of traumatic shock almost as a routine measure, and until recently without much consideration for the possible detrimental effects on the circulation (4). The purpose of the present investigation was to determine whether morphine was harmful or beneficial to dogs in a state of shock. These observations have demonstrated a number of circulatory effects of morphine that are somewhat different from those seen in normal dogs.

**METHODS AND PROCEDURES.** The experiments were carried out on 28 normal dogs ranging in weight from 6.3 to 16.4 kilos. Observations were made on normal dogs in hemorrhagic shock produced by the method of Walcott (5) and on animals in traumatic shock which was produced by muscle contusion as described by Gregersen and Root (6).

Morphine sulfate, dissolved in 2 cc. of isotonic saline, was administered intravenously in a dose of 2 mgm. per kilo. In the hemorrhage experiments the drug was injected about 1 hour after the bleeding, and in the trauma experiments, 2 to 3 hours after the injury.

Oxygen consumption and pulmonary ventilation were measured on the Benedict-Roth spirometer using the Blalock mask. The percentage of oxygen in the bell of the spirometer was always kept above 20 per cent.

Arterial samples were drawn from the femoral artery into syringes which had been previously rinsed with heparin. Simultaneously, mixed venous samples were taken from the right auricle by means of a catheter inserted through the external jugular vein. The position of the catheter was checked at autopsy. The oxygen content of the samples was determined by the method of Roughton and Scholander (7), the arterial carbon dioxide by the method of Van Slyke and Neill (8). Cardiac output was calculated from the arterial venous oxygen difference and the oxygen consumption (Fick principle). Plasma protein concentration was determined using the Abbe refractometer (9), the hematocrit value by centrifugation at 3000 r.p.m. for 30 minutes. The mean blood pressure

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was measured with a mercury manometer and arterial puncture. The peripheral resistance was calculated from the usual formula (10).

*Normal dogs.* Five animals were studied. The results of a typical experiment are shown in figure 1a and certain data from all experiments are combined in figure 3a. Immediately after the injection of morphine the animals showed various degrees of excitement, including muscular activity and hyperventilation.

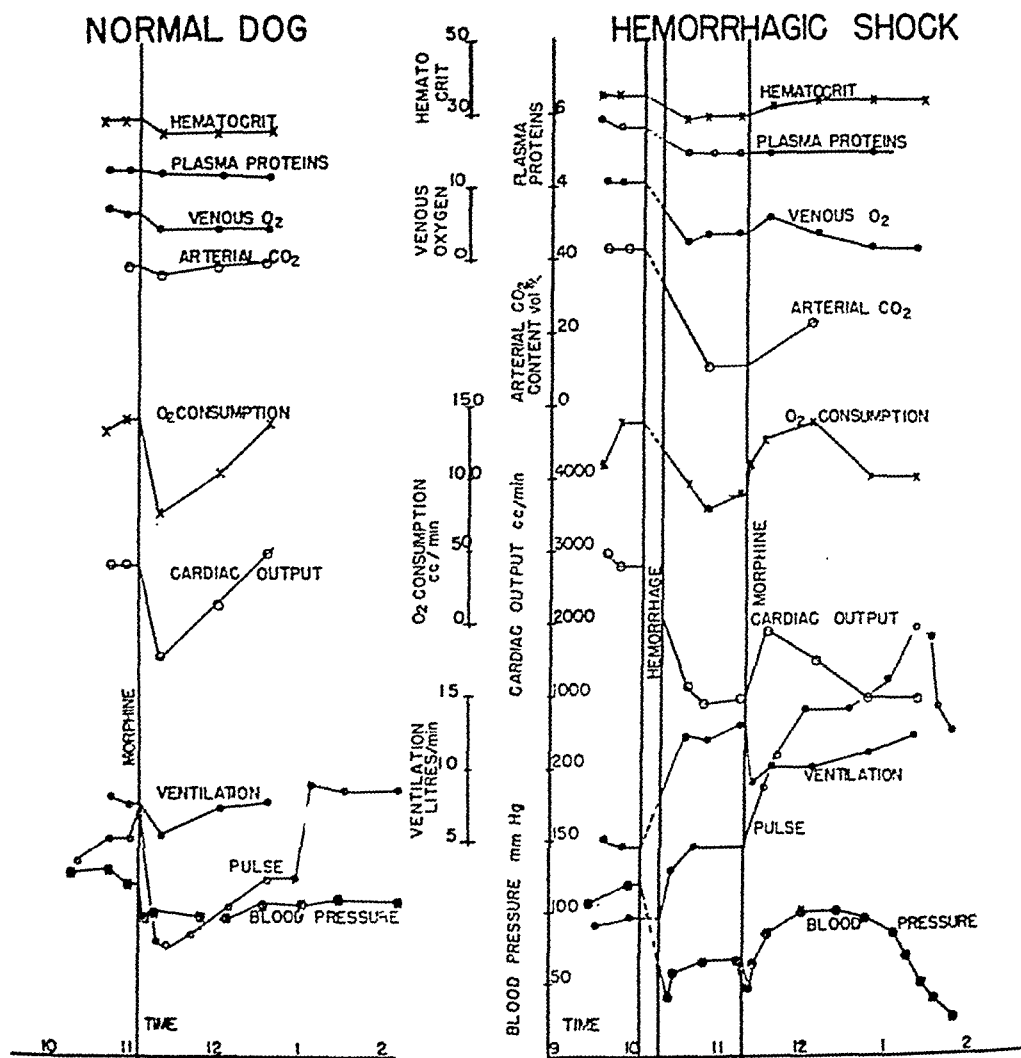


Fig. 1. Typical experiments showing the effects of intravenous morphine on: a, normal dog; b, dog in hemorrhagic shock.

In one to two minutes this was followed by the typical central nervous depression. The respiratory rate and volume, the oxygen consumption, the heart rate and blood pressure fell sharply (fig. 1a). Concurrently, there was a decrease in the venous oxygen, arterial carbon dioxide and the cardiac output; whereas the peripheral resistance increased (fig. 3a). The maximum effect occurred almost immediately, after which there was a gradual return to control conditions. It may be seen from figure 1a that the hematocrit and plasma protein values remained

unchanged. From this it may be concluded that the morphine had no effect on the blood volume.

*Hemorrhagic shock.* Ten animals were studied. The injection of morphine into dogs in hemorrhagic shock caused an abrupt, momentary fall in the blood pressure followed by an immediate rise. The latter was accompanied by a rise in the venous oxygen, arterial carbon dioxide and cardiac output (fig. 1b).

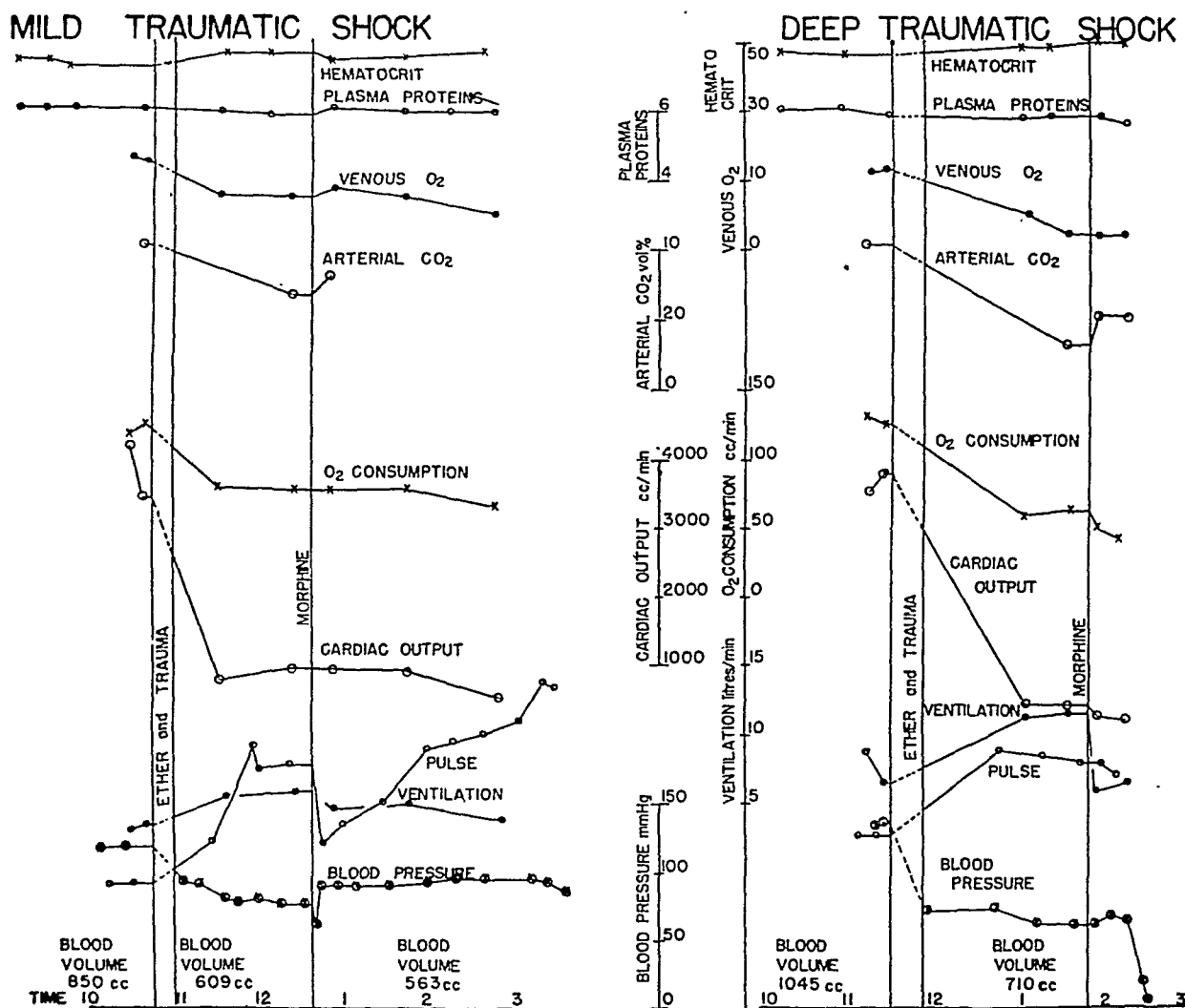


Fig. 2. Typical experiments showing the effects of intravenous morphine on: a, dog in mild traumatic shock; b, dog in deep traumatic shock.

It will be noted that these values approached, but did not reach normal. These effects of morphine are the reverse of those observed in the normal dog. They would appear to be beneficial, but the survival time of this group of animals did not differ appreciably from that observed by Walcott in his untreated dogs (5).

*Traumatic shock.* Of the 13 dogs that were studied, only 9 were suitable for our purpose, as the other 4 died before the effects of the morphine could be observed. Following the injection of morphine in dogs in deep shock, there

was a further drop in the blood pressure, oxygen consumption and cardiac output (see figs. 2 and 3, and table 1). These changes are evidently different from those observed in hemorrhagic shock. Two of the traumatized dogs that were in a much less severe state of shock showed no very definite change after morphine

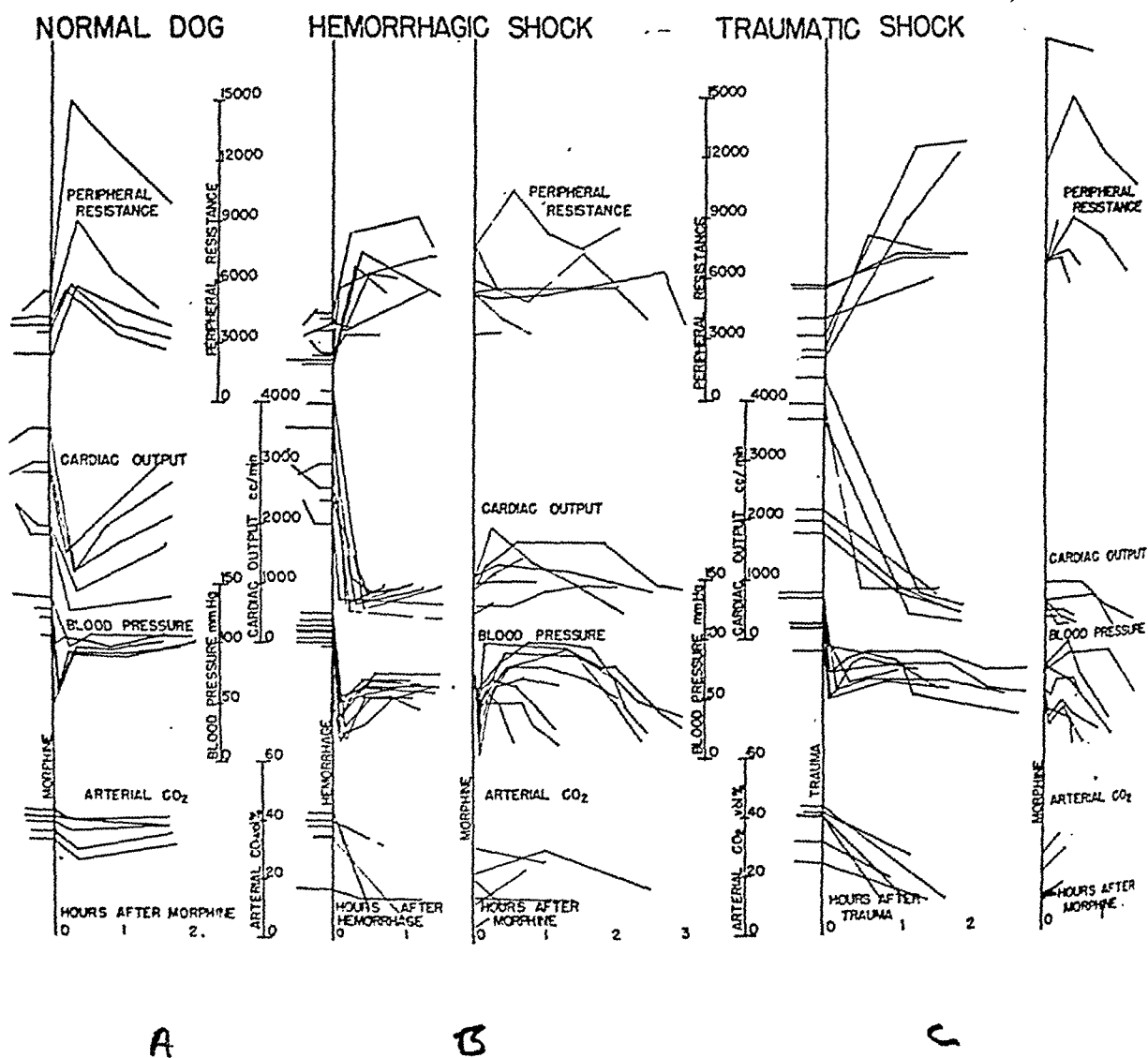


Fig. 3. Composite graph showing blood pressure, cardiac output, peripheral resistance and arterial CO<sub>2</sub> values in all dogs in all 3 series of experiments. The graph was constructed by superimposing the data from all of the experiments in each series on the same time scale. The time scale is constructed so that the shocking procedure is at zero time and the injection of the morphine is also given at a zero time.

(see fig. 2a) except that in one of these where the blood volume was determined again after the morphine the volume was found to be significantly decreased. It is probable that the peripheral vasodilatation (1) and slight increase in blood pressure caused further leakage into the damaged area (13).

DISCUSSION. These experiments indicate that dogs in hemorrhagic shock

and dogs in traumatic shock differ somewhat in their response to morphine, although as is indicated by the criteria in table 1, the dogs were in essentially the same degree of shock before the injection. Experiments undertaken by Wang et al. (11) and Wang (12) indicate that there is a nervous factor in traumatic shock that is not present in hemorrhagic shock. The variations in response to morphine offer further evidence that a fundamental difference in the type or degree of shock may exist in the two groups, as has been noted elsewhere (14). It will be seen from figure 3 that at comparable blood pressures, the peripheral resistance is higher after muscle trauma than after hemorrhage. It is interesting that in all three series of experiments on the normal, the hemorrhaged and the traumatized dogs, the peripheral resistance increased following the injection of morphine, but that this effect was of short duration.

TABLE 1

*Percentage change from control values for all data in traumatic and hemorrhagic shock series. Part a gives the average per cent change from the control values produced by the shock procedure. Part b gives the average per cent change from the shock values produced by the injection of morphine. Averages were made from the values of maximum change.*

	B.P.	H.R.	HEMAT.	PL. PR.	RESP. RATE	VENT.	ART. CO <sub>2</sub>	ART. O <sub>2</sub>	VEN. O <sub>2</sub>	AVO <sub>2</sub> DIFF.	O <sub>2</sub> CON- SUMP.	CARD. OUT.	BLOOD LOSS PER KGM.
<i>a Before morphine</i>													
Hemorrhage....	54	200	85	82	130	180	46	83	26	319	72	29	cc. 37.6
Trauma.....	50	252	102	89	121	162	32	90	37	279	72	30	25.4
<i>b After morphine</i>													
Hemorrhage....	71	242	91	84	115	162	54	86	38	276	100	44	
Trauma.....	53	255	106	87	151	80	38	108	37	269	51	26	

The effect of morphine on the normal dog has been studied by many investigators and the results are in agreement with our observations (1, 2, 3, and others). However, the influence of morphine on the cardiac output has not been previously measured. In the normal dog it causes a rapid fall in the cardiac output and this is roughly proportional to the decrease in the oxygen consumption. At the same time there is a rise in the calculated peripheral resistance. This was completely unexpected in view of the peripheral vasodilatation produced by the morphine. It is clear from these observations that even a normal circulation is markedly affected by the use of intravenous morphine. It should also be emphasized that the small dosage used in the present experiments was only 1/100 of the lethal dose.

The use of morphine in traumatic shock may produce unfavorable changes that influence the fate of the animal. The experiments do not however warrant any conclusion about the mechanism of the difference in response in hemorrhagic and traumatic shock.



## SUMMARY

The intravenous injection of 2 mgm. per kilo of morphine into 5 unanesthetized normal dogs produced a period of excitement after which the respiratory rate and volume,  $O_2$  consumption, heart rate and blood pressure fell sharply. Concurrently, the venous  $O_2$ , arterial  $CO_2$  and cardiac output decreased, whereas the calculated peripheral resistance increased.

The injection of the same dose of morphine into 10 dogs in hemorrhagic shock resulted in a momentary fall followed by a rise in blood pressure. The latter was associated with a temporary increase in venous  $O_2$ , arterial  $CO_2$  and cardiac output.

When morphine was administered to 9 dogs during severe traumatic shock, there was a further reduction in blood pressure,  $O_2$  consumption and cardiac output.

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# PLATELET AGGLUTINATION AND VASOCONSTRICTION AS FACTORS IN SPONTANEOUS HEMOSTASIS IN NORMAL, THROMBOCYTOPENIC, HEPARINIZED AND HYPOPROTHROMBINEMIC RATS<sup>1,2</sup>

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Despite the importance of spontaneous hemostasis, there is considerable speculation and disagreement concerning the means whereby bleeding is arrested. It has been established by direct observation of injured blood vessels that the blood platelets agglutinate at the site of vascular injury (4, 14), and that local vasoconstriction occurs. This constriction is more pronounced in arteries than in veins (14, 21), and is not prevented by denervation (6). It has been suggested but not demonstrated that vasoconstrictor substances liberated from the agglutinated blood platelets (for references, see 36) produce the local vascular contraction (12, 26, 30). These substances do not constrict the minute vessels of the human skin and therefore cannot aid in arresting bleeding from them (26). It seemed of interest, however, to determine whether they play a part in hemostasis in larger blood vessels. Vasoconstriction alone, unaccompanied by platelet agglutination, can arrest bleeding from severed arteries (21), but both processes usually contribute to hemostasis (23, 31). However, Macfarlane (20) has recently stated that constriction plays the major rôle in the arrest of capillary bleeding and that platelet agglutination is not essential. New evidence which demonstrates that the capillaries are unable to contract (5, 7) has made a further investigation of capillary hemostasis desirable. Two other factors which are believed to aid in arresting hemorrhage are fibrin deposition and clot retraction (20, 25). This belief is based upon very little concrete evidence, hence a re-examination of the rôles of these factors in hemostasis seemed desirable. Pathological bleeding may occur in animals with thrombocytopenia or with hypo-coagulable blood, but in only one study in which dicumarol was used (6) has hemostasis in such animals been studied by direct observation of cut vessels.

From this brief summary, it is evident that many questions concerning the mechanism of hemostasis remain unanswered. The present study was undertaken to elucidate this mechanism by observing the changes which follow incision of blood vessels in normal animals, and by making a similar study in animals given antiplatelet serum, heparin or dicumarol.

<sup>1</sup> Aided by a grant from the Baruch Committee on Physical Medicine to Columbia University.

<sup>2</sup> A preliminary report of this work has been published in the Federation Proceedings 5: 117, 1946. This work was presented before the American Congress of Physical Medicine, New York City, September, 1946.

**METHODS.** Experiments were performed either on venules in the mesoappendix or on large branches of the superior mesenteric arteries and veins of 80 to 100 gram white rats anesthetized with nembutal. Many animals were employed for only one experiment. Others were used for experiments on two vessels of different types, situated a considerable distance apart. The mesoappendix was arranged in the manner suggested by Chambers and Zweifach (5) except that the tissue was covered by a rigidly fixed coverslip over which Ringer's solution flowed. Gelatin was omitted from this solution since it agglutinates extravascular red blood cells. The venules were transected with a splinter of razor blade mounted on a micromanipulator. This was introduced beneath the tissue, elevated to press the tissue against the coverslip, and moved horizontally to cut the desired vessel. The coverslip to some extent prevented the hemorrhage from obscuring the field but permitted a free flow of blood from the underside of the mesoappendix. In the experiments on the mesenteric vessels, several centimeters of small intestine were extruded and placed around a ring of plastic material to spread out the mesentery. A nick was made in the artery or vein with a scissors which penetrated one wall but did not transect the vessel completely. The flow of Ringer's solution removed the blood so that the vessels remained visible. The fat surrounding the vessels and the speed of flow often made it impossible to detect intravascular blood flow.

The blood vessels were studied at magnifications of 100X and 26X, and their size was measured with a calibrated ocular micrometer disc. The diameter in micra of the vessels studied was: mesoappendiceal venules, 9.5 to 40, mesenteric arteries, 79 to 210, average 170, mesenteric veins, 135 to 310, average 220. The occurrence of bleeding and the degree of vasoconstriction were recorded at frequent intervals for at least half an hour after the vessel was incised, or until the death of the experimental animal. The data is summarized in table 1.

**RESULTS.** *Rats with normal vessels.* The events which occurred when a small (9 to 22  $\mu$ ) venule of the mesoappendix was transected depended on the nature of the cut. When the blade was dull, the stump of the vessel was sometimes immediately occluded so that no bleeding occurred. Since connective tissue fibers obscured the tip of the stump, it could not be determined whether bleeding was prevented by tension of connective tissue fibers which had been pulled across the vessel, or whether the endothelium on one side of the venular stump adhered to that on the other. In some instances, stasis<sup>2</sup> occurred rapidly in these stumps. The caliber of the venules apart from the tip of the stump was unchanged. In the majority of experiments, and in all of those included in table 1, the venules were transected with a sharp blade. When this was done, the thin mesoappendix gaped, and the vessel bled from both its central and peripheral ends. Within one minute, colorless, refractile bodies about 6 micra in diameter could be seen at the tip of the bleeding stumps, but not within the vessel lumen (fig. 1). Subsequent experiments (see below) demonstrated that these bodies were formed from blood platelets, and they will be referred to as

<sup>2</sup> The term stasis denotes circulatory arrest brought about by packing of red blood cells which is probably a consequence of loss of plasma through the vessel wall (17).

platelet plugs. The plugs did not at first cause hemostasis for blood continued to flow from the vessel in gradually decreasing amounts. Bleeding ceased in an average of 4 minutes, either from both ends at approximately the same time or first from the peripheral end. The vessel walls near the tips of the stumps were obscured by the platelet plugs. Nevertheless, the diameter of the vessel at this site could be estimated since the lumen was filled with red cells. Constriction

TABLE 1

*Experiments on transected mesoappendiceal venules and on nicked mesenteric arteries and veins*

VESSEL	PREPARATORY TREATMENT	TOTAL NO. OF EXPTS.	FREQUENCY OF RESULTS							Contraction of uninjured vessel
			Platelet plug present	Cessation of bleeding	Recurrence of bleeding				No final cessation of bleeding	
					None	Once	Twice	More than twice		
Venule...	None	8	16*	16*	2*	2*	4*	8*	0	
Vein.....	None	15	15	15	6	5	2	2	0	11
Artery....	None	3	3	3	0	2	0	1	0	3
Vein.....	Chronic S	3	3	3	2	0	0	1	0	3
Vein.....	Chronic S & C.....	4	4	4	1	1	0	2	0	4
Artery....	Chronic S & C	4	4	4	0	0	2	2	0	4
Vein.....	Acute S & C	3	3	3	0	0	0	3	0	3
Artery....	Acute S & C	3	3	3	0	0	1	2	0	3
Venule...	Purpura	3	0*	1*†	1*†				5*	
Vein.....	Purpura	5	0	0					5	0
Artery....	Purpura	3	0	0					3	0
Vein.....	Heparin 500 u/kgm.	10	9	6	1	2‡	0	3§	7	7
Vein.....	Heparin 2250 u/kgm.	4	2	0					4	2
Vein.....	Dicumarol	5	5	5	0	0	2	3	0	5
Artery....	Dicumarol	1	1	1	0	0	0	1	0	1
Vein.....	Dicumarol and low vit. K diet	3	3	1	0	1‡	0	0	3	3

\* Each end of transected venule counted separately.

† Hemostasis occurred by stasis.

‡ Bleeding recurred shortly after its initial cessation and continued for over 20 minutes until the experiment was terminated.

§ In one case, bleeding recurred shortly after its initial cessation and continued for over 20 minutes until the experiment was terminated.

S = Splanchnic nerves sectioned below the diaphragm. S&C = Splanchnic nerves sectioned and coeliac ganglion removed.

did not occur in any part of the cut venule. In most cases, bleeding recommenced during the course of the experiment, although the platelet plugs remained in place. The hemorrhage recurred from either end of the severed vessel from 1 to 12 times and each recurrence lasted for about a minute. In a few instances, stasis was observed in either stump some minutes after the vessel was cut, but this did not prevent a recurrence of hemorrhage.

Venules of larger caliber (30 to 40  $\mu$ ), when cut with a sharp blade, behaved

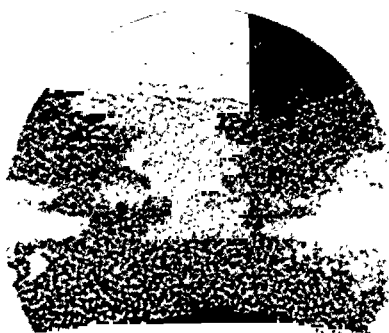
1



2



3



4



5

Fig. 1. Transected non-muscular venule. A platelet plug is seen at the tip of one stump at the right hand border of the hole in the mesoappendix. This stump is not visible since it no longer contains red blood cells. Blood is still flowing from the other stump at the tip of which a platelet plug is forming. One scale division is equal to 50 micra in this and subsequent figures. All figures are un-retouched except for scale markings.

Fig. 2. Mesenteric artery (top) and vein of normal rat.

Fig. 3. The same vessels as in figure 2 nine minutes after the vein was nicked. Bleeding ceased in  $1\frac{1}{4}$  minutes and was renewed three times. The last recurrence took place five minutes after incision. A platelet plug is seen lying on the vein, and local constriction of both artery and vein is apparent.

Fig. 4. Sympathectomized artery (top) and vein 20 minutes after the artery was nicked. Bleeding was arrested in  $2\frac{1}{4}$  minutes and recurred twice. The second recurrence took place 19 minutes after incision. Note the platelet plug and marked local constriction of both blood vessels.

Fig. 5. Mesenteric vessels of purpuric rat (platelet count 12,000)  $1\frac{1}{2}$  minutes after the artery (below) was nicked. Note the contraction of the artery, the continuous stream of blood, and the absence of a platelet plug and of venous constriction. Conditions remained essentially unchanged until the animal's death 29 minutes after incision.

like small venules except that the cut central end, and in one instance the peripheral end constricted 45 seconds to 5 minutes after transection. The stumps resumed their control diameters 9 minutes or less after incision. Their relaxation did not produce a recurrence of hemorrhage.

In order to investigate the importance of vasoconstriction in the hemostatic response, the mesenteric arteries and veins were studied. These vessels contain abundant smooth muscle, as determined by their histological appearance and pharmacological behavior. Both the artery and vein were constricted by one or more of the vasoconstrictor substances in the blood platelets. This was evident since the topical application of normal rat serum or of purified platelet substance, SMC (36), produced contraction. That the active material in serum arose from the platelets was demonstrated by the fact that serum from thrombocytopenic rats failed to affect the vessels. The application of 1:330,000 epinephrine constricted the artery only; the vein dilated slightly. This suggests that, whereas a constrictor response to epinephrine may be used to determine the location of smooth muscle in the vessels on the arterial side of the capillary bed (5), it cannot be used for the same purpose on the venous circulation. The mesenteric artery and vein run side by side, so that the response of both the cut vessel and its neighbor could be studied. Thus, it was possible to ascertain whether, during hemostasis, sufficient vasoconstrictor material is liberated from the platelet plug to affect nearby vessels. This could not be determined by observation of the cut vessel, since the mechanical trauma alone induced marked vasoconstriction.

When a mesenteric vessel was nicked, blood immediately flowed from the wound. A local constriction of the injured vessel was apparent in about 15 seconds and reached its maximum within one minute. At this time, the vessel had decreased more than 80 per cent in diameter at the site of the wound, and tapered to its original size over a distance of about 1 mm. Simultaneously, the incision was covered by a refractile body, and bleeding ceased in an average of two minutes. The refractile body was chiefly extravascular, but in some instances, enough of it lay within the vessel to impede or stop the flow of blood. When this occurred, emboli were given off as the circulation was resumed. In 12 out of 18 experiments, hemorrhage recurred, usually only once, at a variable time after the initial cessation of bleeding. During the recurrence, which commonly lasted for less than a minute, blood flowed through or beneath the platelet plug. In 14 experiments, there was narrowing of the adjacent uninjured vessel opposite to and for about 0.5 mm. on either side of the site of injury. This constriction developed more slowly than did that in the injured vessel; it was first evident 20 to 30 seconds after the incision was made, and reached its maximum in 2 or 3 minutes. After bleeding ceased, the constricted segments of both vessels gradually relaxed. The incised vessel never completely regained its normal diameter during the experiment whereas the uninjured vessel frequently did so. Renewed constriction of the uninjured vessel occasionally followed a recurrence of the hemorrhage (figs. 2 and 3).

Studies were made of the refractile body which appeared at the site of bleeding. Smears made within 5 minutes of the formation of the plug and stained with

brilliant cresyl blue<sup>4</sup> demonstrated that it was composed of blood platelets. In sections of the vessels fixed more than one half hour after the incision and stained with hematoxylin and eosin, the plug appeared as a homogeneous eosinophilic mass which lay for the most part on the surface of the mesentery. Material of similar composition was occasionally seen lying within the vessel, and in some instances continuity with the outer mass could be demonstrated through a tear in the vessel wall. Individual platelets were not distinguishable within the mass, which had the appearance of coagulated protein. No fibrin could be detected in the plug in sections stained with phosphotungstic acid hematoxylin.

*Animals with abdominal sympathectomy.* Subdiaphragmatic section of the splanchnic nerves was carried out in rats. In most instances celiac ganglionectomy was also performed. Denervation was believed to be complete since the nerves could be readily identified before section. Neither the administration of CO<sub>2</sub> nor a decrease in carotid sinus pressure produced visible constriction of the mesenteric vessels in normal animals, hence these procedures could not be used to test the adequacy of denervation. Experiments on the mesenteric vessels were performed immediately or from 6 to 19 days after the operation. The slight increase in the duration of bleeding and the greater frequency of renewed hemorrhage (table 1) which were the only observed changes in the hemostatic mechanism produced by abdominal sympathectomy, have no statistical significance (fig. 4).

*Heparinized animals.* Preliminary experiments patterned after those of Solandt and Best (29) were performed to ascertain the approximate dose of heparin required to prevent thrombosis in shunts placed in the circulation. Large rats were injected intravenously with 500 or 2250 units of heparin per kgm. Thirty to 45 minutes later, circulation was started through a glass U-tube connected by transflex plastic tubing<sup>5</sup> to cannulae in the abdominal aorta and vena cava. When the flow through the shunt had been established for 1½ hours, the clotting time was measured on cardiac blood (Lee-White method), and the shunt was examined for thrombi. The data (table 2) indicate that in all but one experiment, blood flow continued through the shunt until the termination of the experiment. After the injection of 500 units of heparin/kgm., small white thrombi were sometimes found on the walls of the plastic and glass tubing. In one experiment, clotted blood was found in the shunt. The administration of 2250 units/kgm. entirely prevented the production of thrombi. In rats which had not received heparin (table 3), circulation through the shunt was arrested within 15 minutes, apparently by a platelet thrombus in the tip of one of the cannulae. The remainder of the shunt contained coagulated blood.

The behavior of nicked mesenteric veins was studied in 14 rats 30 to 45 minutes after heparinization. Ten rats received 500 units/kgm. and 4 were given 2250 units/kgm. Platelet agglutination did not occur at the site of bleeding in one of the rats which had received 500 units/kgm. and in 2 of those given 2250 units.

<sup>4</sup> The author is grateful to Dr. Paul Klemperer of the Mount Sinai Hospital, N. Y. C. for suggesting this method.

<sup>5</sup> Irvington Varnish and Insulator Co.

The rest of the animals produced platelet plugs. However, in 2 rats given 2250 units/kgm. and in 3 animals which received the smaller dose, hemostasis did not occur. Blood continued to flow through or beneath the plugs throughout the entire experiment, and the plugs fragmented easily. In 3 of the animals given 500 units/kgm. although bleeding ceased initially, there were recurrences lasting for over 20 minutes. In one of these experiments, and in a preliminary experi-

TABLE 2

*The occurrence of thrombosis in shunts placed in the circulation of heparinized rats*

DOSE OF HEPARIN	TIME BETWEEN INJECTION & ESTABLISHMENT OF SHUNT	CLOTTING TIME AT END OF EXPT.	WHITE THROMBUS	SITE OF THROMBUS	DURATION OF FLOW THROUGH SHUNT
units/kgm.	min.	hrs.			hrs.
500	35	>6	+	Cannula tip	0.58
500	30	1	+	Mural	
500	35	>5.5	+	Mural	
500	30	>6	0*		>1.5
500	40	>5.5	+	Mural	
500	40	>4.5	+	Mural	
500	45		0		
2250	45	>21	0		
2250	40	>21	0		
2250	35	>6	0		

\* In this experiment only, clotted blood was present in the shunt but did not arrest blood flow.

TABLE 3

*The occurrence of thrombosis in shunts placed in the circulation of normal and thrombocytopenic rats*

CONDITION	PLATELET COUNT PER CU. MM.	PURPURA	WHITE THROMBUS*	SITE OF THROMBUS	DURATION OF FLOW THROUGH SHUNT
					hrs.
Normal.....		0	+	Cannula tip	0.17
Normal.....		0	+	Cannula tip	0.05
Thrombocytopenic.....	386,000	0	+	Cannula tip	0.25
Thrombocytopenic.....	122,000	0	0		>1.5
Thrombocytopenic.....	74,000	+	0		>1.5
Thrombocytopenic.....	44,000	+	0		>1.5

\* In all experiments, the shunt contained clotted blood.

ment not included in the table, the plug became detached from the tissue and was not replaced. The results indicate that the platelet clumps which formed in heparinized animals differed qualitatively from those produced in normal animals, but histological studies failed to demonstrate any difference between them. The cut vein contracted in all experiments. Local constriction of the uninjured artery, however, occurred only when a platelet plug was present.



*Animals with low prothrombin levels.* The prothrombin time (Quick method) of rats was increased from a normal value of 19 to 20 seconds to values ranging up to 34 seconds by the oral administration of dicumarol. Hemostasis was normal in these animals except for an increased incidence of renewed bleeding which was of no statistical significance. The longest prothrombin time in this series of experiments represents a prothrombin level of slightly over 20 per cent of normal when calculated from a dilution curve of rat plasma similar to that constructed by Quick (25).

In order to obtain lower prothrombin levels, 3 rats were maintained for 10 days on a diet low in vitamin K (24) and were given 10 to 20 mgm. of dicumarol on each of the last 3 to 5 days. At the end of this period, the prothrombin times were longer than 300 seconds. Platelet clumps were present in all three animals after the mesenteric veins had been nicked. Nevertheless, bleeding did not cease in 2 rats, and was only temporarily arrested in the third. Local constriction of both the cut vein and the uninjured artery was observed in every instance.

*Purpuric animals.* Thrombocytopenic purpura was produced in rats by the injection of antiplatelet serum (37). The animals were used for experimentation on the day following injection. Platelet counts on these rats averaged 83,500/cu. mm. (range 12,000 to 273,000) as compared with the normal value of 800,000/cu. mm. (13). Most of the bodies counted as platelets were small and poorly defined, unlike the platelets seen in the blood of normal animals. Well-defined platelets were not seen in Wright-stained blood smears. When aortic-vena caval glass shunts were placed in the circulation of purpuric rats (table 3) no white thrombi were formed. Although the shunts contained some coagulated blood, circulation continued through them for the duration of the experiment ( $1\frac{1}{2}$  hrs.).

When the mesenteric vessels or the venules of the mesoappendix were cut in purpuric animals, no platelet clumps were seen at the site of injury in the living animal, nor were they observed in fixed material. Bleeding continued without cessation until the animal died of exsanguination or until the experiment was terminated. The cut mesenteric vessel contracted in the usual fashion, but a localized constriction of the uninjured vessel was never observed (fig. 5). In some experiments, the artery and vein eventually narrowed throughout their entire length. This was undoubtedly a part of the generalized vasoconstriction which is known to occur after hemorrhage.

**Discussion.** The incision of a vessel is usually rapidly followed by the local production of a clot<sup>6</sup> composed of blood platelets (4, 14, 31). Microscopic examination shows that the platelets composing the plug undergo rapid fusion. This is in agreement with the results of Best, Cowan and Maclean (3). Fibrin is not seen in stained sections of the plug fixed one-half hour after its formation. Many other investigators have similarly failed to detect fibrin in fresh intravascular platelet thrombi, although fibrin, as well as red and white blood cells, is subsequently added (1, 11, 33). Since the platelet plugs seen in our experiments

<sup>6</sup> In accordance with Copley's suggestion, the term clot is used to include both blood coagulation and cell agglutination.

are for the most part extravascular, it is unlikely that fibrin is added after their formation. Under more physiological conditions, extravasated blood probably coagulates once bleeding has been initially arrested by a platelet plug and vasoconstriction. In the experiments reported here, coagulation does not occur in the immediate vicinity of injured vessels because the flow of Ringer's solution or the presence of a coverslip prevents the accumulation of blood. The absence of a fibrin clot may explain the surprisingly frequent recurrences of hemorrhage. Since these recurrences are observed when a coverslip is interposed between the vessels and the stream of Ringer's solution, they cannot be attributed to a mechanical disturbance of the platelet plug by the flow of fluid.

Quick (25) believes that clot retraction plays a fundamental rôle in hemostasis by drawing together the edges of the severed vessel. No published data have been encountered to support this contention, and the absence of demonstrable fibrin from the platelet plug which fills the vascular defect makes the hypothesis unlikely. When a blood coagulum fills the wound tract, syneresis may aid in narrowing the wound.

Vasoconstriction occurs at the site of hemorrhage from many large blood vessels. According to some investigators (14, 21) this response is more pronounced in arteries than in veins but in the rat mesentery, marked contraction was seen in both types of vessel. The constriction is not mediated through nerves since it is present in denervated arteries in the ears of rabbits (6), and in the mesenteric vessels of rats subjected to abdominal sympathectomy. The rapid appearance of constriction in incised rat mesenteric vessels, and its occurrence in the absence of a platelet plug indicate that it is not produced by the vasoconstrictor substances in blood platelets although these substances may prolong and perhaps augment the response. The contraction, therefore, appears to be a response of vascular smooth muscle to the mechanical stimulus of incision. The experiments reported here indicate that vasoconstriction alone does not arrest bleeding from the arteries or veins of the rat mesentery. Thus hemostasis fails to occur in purpuric and heparinized animals which do not form platelet plugs, although constriction of the injured vessel appears to be as pronounced as in normal animals. Persistent and extreme spasm has, however, been shown to arrest arterial bleeding without the formation of a clot, especially when the vessel is transected rather than punctured (6, 21, 31). Under these circumstances, hemostasis may be of short duration (6) and occurs only when the blood vessel is not overheated (31).

It has been suggested that the vasoconstrictor substances which are liberated from the blood platelets during their breakdown (for references, see 36) aid in producing the vasoconstriction which occurs during hemostasis (12, 26, 30). Reid (26) found that intradermal injections of human serum, serum ultrafiltrate or an extract of blood platelets failed to produce blanching of the skin. From this, he concluded that the platelet substances are without effect on skin capillaries and that they therefore cannot assist in arresting bleeding from these vessels. The experiments on rats reported here constitute the first evidence of the activity of the vasoconstrictor substances in the blood platelets *in vivo* and

indicate that they play a part in hemostasis in contractile vessels. Incision of a muscular mesenteric vessel is followed not only by a contraction of the cut vessel, but usually also by a local constriction of the uninjured artery or vein adjacent to the site of bleeding. This constriction never occurs in the absence of a platelet plug although it is almost invariably seen when a plug is present at the site of injury. It is initiated later and reaches its maximum more slowly than does the constriction of the cut vessel. After the uninjured vessel has relaxed, it may contract a second time in the absence of further mechanical stimulation if bleeding is renewed. These observations indicate that this constriction is not the result of the trauma of the incision. Nor is it reflex in nature, since it occurs in animals which have undergone abdominal sympathectomy. The response of the vessels of both normal and purpuric rats to topically applied purified platelet extract, SMC (36), or rat serum demonstrates that the active substances in the blood platelets are able to contract both the arteries and veins, and that they readily diffuse through the tissues surrounding the vessels. Thus it appears that the platelet substances are responsible for the contraction of the uninjured vessel described above. The contraction of the injured vessel is primarily the result of mechanical stimulation of the vascular smooth muscle. However, the observation that constriction of punctured arteries is more enduring when a large clot lies on the wound (6) suggests that the platelet substances prolong and perhaps augment the response of the injured vessel. This humoral factor is of less importance in hemostasis than is the vasoconstriction induced by direct muscle stimulation or the physical action of the platelet plug.

In non-muscular blood vessels, bleeding is arrested by a somewhat different mechanism than in larger muscular vessels. Capillaries in the frog's tongue do not contract when cut and are prevented from bleeding by stasis and by adherence of the endothelium which closes off the stump (16). Stasis, unaccompanied by contraction, prevents the outflow of blood from cut mammalian mesenteric capillaries (31). However, Magnus (22) states that frog capillaries as well as arteries and veins contract when punctured. In the small venules in the rat mesoappendix, constriction is not observed following transection. This is not surprising since Chambers and Zweifach (5) have shown that these vessels are non-muscular. When a venule is transected with a dull blade, bleeding usually fails to occur. It is not clear whether the escape of blood from the vessel is prevented by adherence of the endothelium or by the tension of connective tissue fibers drawn about the stumps. Stasis usually occurs in the stumps soon after transection. When a sharp blade is employed, both stumps bleed and hemostasis is produced by a platelet plug, with occasional concomitant stasis. Incessant bleeding occurs from transected venules in thrombocytopenic rats, which fail to form platelet plugs, except that in one experiment the flow of blood was arrested by stasis. Some investigators have concluded that constriction is the primary means whereby capillary bleeding is arrested (15, 20, 22). Their conclusion is based on observations of punctured capillary loops at the base of human finger nails. Unfortunately, the walls of these vessels are not visible. Vasoconstriction is presumed to be present when red blood cells cannot be seen in the punc-

tured loops. Heimberger (15) has described in detail the changes which follow puncture of a loop. These changes are more readily explained as the result of the local production of a platelet thrombus than as a consequence of local vasoconstriction.

Although the administration of large doses of heparin either diminishes or prevents the formation of platelet thrombi in glass shunts inserted in the circulation of rats, platelet plugs are often formed at the site of incision of mesenteric veins. The quality of these plugs, however, indicates that the adhesiveness of the platelets is reduced by heparin. Bleeding usually continues through the plugs, and clumps of platelets or the entire plug may become detached. A similar decrease in the adhesiveness of the platelets was observed in human heparinized blood studied *in vitro* (34) and in the thrombi formed in dogs given insufficient heparin to prevent entirely the production of thrombi (9). It seemed possible that an absence of fibrin was responsible for the friability of the plugs produced in heparinized rats, since some investigators believe that platelets adhere to one another by means of a layer of fibrin. However, the fact that no fibrin is demonstrable in the plugs formed in normal rats makes this hypothesis unlikely. No previous experiments have been reported in which the blood vessels of heparinized animals have been observed following incision. Copley and Lalich (8, 18) have reported that very large subcutaneous injections of heparin sometimes raise the bleeding time of mice. More often, an increased incidence of renewed bleeding is observed which either occurs spontaneously or follows the application of a tourniquet. Macfarlane (20) states that heparin does not increase the bleeding time, but he does not report the dose used. Since vascular injury is such a powerful stimulus to platelet agglutination, it is apparent that very large doses must be employed to impair hemostasis.

The administration of large doses of dicumarol raises the bleeding time of mice, and in 30 per cent of the animals increases the incidence of renewed bleeding (18, 19). The drug also prevents the normal formation of clots when the auricular blood vessels of rabbits are punctured. Bleeding from the artery ceases only for the duration of vasoconstriction (about 10 min.) and bleeding from the vein is not even temporarily arrested (6). The results of experiments on rat mesenteric vessels indicate that hemostasis is normal if the prothrombin level is higher than 20 per cent of the normal value, which is in agreement with the conclusion of Quick (25). When the prothrombin level is below this value, platelet plugs are formed but fail to act as effective hemostatic agents. It appears that, like heparinization, depression of the blood prothrombin level affects hemostasis by impairing the ability of the platelets to agglutinate. Diminished platelet agglutination in glass cells *in vivo* (9) and decreased platelet adhesiveness *in vitro* (35) have previously been shown to follow the administration of dicumarol.

In experimental thrombocytopenic purpura, the bleeding time is markedly prolonged (2, 32). Incised blood vessels of purpuric rats bleed for over one-half hour or until the animal dies of exsanguination. Microscopic observation of these vessels demonstrates the complete absence of platelet plugs at the site of injury. This probably explains the hemorrhagic tendency of these animals.

It may seem surprising that not even a small platelet plug is formed since the platelet count indicates the presence of circulating blood platelets. However, these platelets have lost their capacity to take Wright's stain and to agglutinate. This was shown by the failure of platelet thrombi to form in glass shunts placed in the circulation of purpuric rats and by the absence of well-defined platelets from blood smears.

The results reported here permit renewed speculation on the factors involved in the arrest of bleeding from puncture wounds of the skin such as those produced in the determination of bleeding time. The formation of a platelet plug at the site of bleeding appears to be of primary importance in the arrest of hemorrhage from small vessels. Hence it is not surprising that the bleeding time is increased in patients with severe thrombocytopenia (10). However, there may be a lack of correlation between the platelet count and bleeding time (20). A hemorrhagic tendency in the presence of a normal platelet count may indicate that, as in thrombocytopenia produced by antiplatelet serum, the remaining platelets have a diminished capacity for agglutination which impairs their hemostatic function. Reference to measurements of platelet adhesiveness in thrombocytopenia has not been found. Schultz (28) and other investigators have stressed the importance of factors other than the platelets in hemostasis. The results presented here as well as those of others indicate that endothelial stickiness and tissue tension, as well as stasis, can arrest bleeding from small vessels. Hemostasis produced by these means may account for the normal values of bleeding time sometimes obtained in thrombocytopenic individuals (20, 27). From a consideration of the lack of correlation between the bleeding time and the platelet count, and from a study of the capillary loops at the base of the nail, Macfarlane (20) has concluded that platelet agglutination is unimportant in hemostasis, and that contraction, followed by coagulation, is the primary means by which bleeding from capillaries is arrested. His conclusions are not supported by the majority of studies in which the capillary wall can be seen.

#### SUMMARY AND CONCLUSIONS

The mechanism of hemostasis was investigated in rats by microscopic observation of the local responses which followed incision of mesoappendiceal venules and large branches of the mesenteric arteries and veins. The results are tabulated in table 1.

Cut normal or sympathectomized mesenteric vessels contract locally, primarily as a consequence of traumatic stimulation of the vascular smooth muscle. The rapid formation of a plug of agglutinated platelets at the site of injury is followed by cessation of bleeding. Vasoconstrictor substances liberated from the platelets induce contraction of the uninjured blood vessel lying adjacent to the nicked vessel and probably contribute to the contraction of the incised vessel (figs. 2, 3 and 4). Bleeding frequently recurs for brief intervals after its initial cessation. This can perhaps be attributed to the experimental conditions which prevent the formation of a fibrin clot in the wound.

Non-muscular venules do not contract when cut. When a dull blade is used,

hemorrhage from the stump is usually prevented, probably by adherence of the endothelium or by pressure of connective tissue fibers, often accompanied by stasis. Transection with a sharp blade is followed by bleeding which is arrested by the formation of a platelet plug (fig. 1). In these instances, bleeding frequently recurs for brief periods.

In some animals given large doses of heparin, no platelet plug forms at the incision and bleeding is incessant. In other heparinized animals, and in rats in which the prothrombin level is markedly lowered by the administration of dicumarol, plugs are produced which are ineffective in arresting bleeding. The incised vessel in all animals contracts normally, but the uninjured vessel contracts only in the presence of a platelet plug. Heparin and dicumarol administration impair hemostasis by diminishing the ability of the platelets to agglutinate.

Rats with thrombocytopenic purpura induced by the injection of antiplatelet serum fail to form platelet plugs, and bleeding is not arrested. Although contraction of the injured vessel occurs, the adjacent uninjured vessel fails to contract since no platelet plug is present as a source of vasoconstrictor substances (fig. 5). The platelets apparently present in the circulating blood are incapable of adhering either to the walls of a shunt inserted in the circulation or to the site of vascular injury.

The mechanism of hemostasis in normal and pathological conditions is discussed, with reference to the measurement of the bleeding time.

**ADDENDA.** In this paper, the term platelet agglutination refers to the formation of hemostatic plugs and of white thrombi, since both are composed almost entirely of blood platelets. This agglutination, as well as the adhesiveness of platelets to glass *in vitro* (34) is diminished or prevented by heparin. However, the fact that heparin may decrease the platelet count and *produce* small platelet clumps *in vivo* as well as *in vitro* (Copley and Robb, *Amer. J. Clin. Path.*, 12: 416 and 563, 1942, and Jaques, personal communication) suggests the occurrence of other types of platelet agglutination.

The suggestion that poor hemostasis in patients with normal or moderately reduced platelet counts may be attributed to impaired platelet agglutination is supported by the studies of Jürgens (*Deutsch. Arch. f. klin. Med.*, 172: 248, 1931).

Since this paper was sent to press, the detailed investigations of Apitz (*Virchow's Arch.* 108: 540, 1942, and *Ztschr. f. d. ges. exp. Med.*, 111: 540 and 554, 1942) have come to my attention. His observations and conclusions following *in vivo* and histological studies of hemostasis in normal and heparinized animals are in almost complete accord with mine. Differences in our findings are: (1) Apitz concludes from histological sections of superficial incisions of the liver that capillary bleeding is arrested by fibrin deposition rather than by the formation of platelet plugs. (2) He does not refer to the vasoconstrictor substances in the blood platelets. (3) He mentions the fact, inadvertently omitted from this paper, that the stumps of transected, plugged vessels often contain only plasma, and points out that this phenomenon might simulate constriction in studies of vessels such as the nailfold capillaries whose walls cannot be seen.

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# THE CONTRIBUTORY RÔLE OF THE AFFERENT NERVOUS FACTOR IN EXPERIMENTAL SHOCK: SUBLETHAL HEMORRHAGE AND SCIATIC NERVE STIMULATION<sup>1,2</sup>

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Although it is well known that the shock syndrome may result from loss of blood volume alone, an increasing quantity of data points to the salient differences in the clinical manifestations of experimental animals suffering from uncomplicated hemorrhage on the one hand and muscle trauma with identical fluid loss on the other. Recently, Wang et al. (1, 2) have reported an experimental study of the loss of effective circulating volume in dogs subjected to graded hemorrhage and have compared the resulting syndrome with that of a second series of animals subjected to trauma. This study not only shows that there is a striking difference in the ability of shocked animals to withstand a specific loss of blood, but also that there are obvious differences in the clinical signs shown by the two groups even when the blood volume reduction is identical. These differences suggest that the sympathetic nervous system is intensely active in traumatic shock. In an endeavor to study and evaluate the nervous mechanisms which might account for the differences between hemorrhage and trauma, the present investigation was undertaken.

There are, in general, two means by which the afferent nervous factor in shock may be studied. One method, which has been used previously by others (3), is to observe the effect of interruption of the pathways carrying afferent impulses from the region of injury. The other, which is described below, consists of combining a sublethal hemorrhage with prolonged stimulation of somatic afferent nerve trunks.

**METHOD.** Experiments were carried out on 30 healthy mongrel dogs ranging in weight from 6 to 16 kgm. Control plasma volume, hematocrit values and serum protein concentrations were determined on each animal on the day preceding the experiment. Plasma volume determinations were made using the dye (T-1824) dilution method (4,5). Hematocrit values were obtained by centrifuging heparinized blood samples in Wintrobe tubes, for one half hour at 3000 r.p.m. The blood volume is calculated from the plasma volume and hematocrit readings, without correction for the small amount of plasma trapped

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<sup>2</sup>A preliminary report of this work appeared in *Fed. Proc.* 4: 54, 1945.

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between cells. The serum protein concentrations were determined refractometrically using an Abbe refractometer (6).

On the day of the experiment, rectal temperature was measured, heart rate was counted and mean blood pressure was determined by puncturing the femoral artery. Ether was then administered to the animal for 15 to 35 minutes during which the sciatic nerves were exposed, sectioned, and shielded electrodes applied to their central ends. The animal was bled according to the procedure described elsewhere (2). Since hemorrhaged animals will generally survive if they have a residual blood volume of 60 cc. or more (including dilution) per kgm. of body weight, we attempted to bleed in such a manner that all of the animals retained at least that amount of blood<sup>4</sup>. Except for 2 instances, all of the dogs reported here did retain this volume. Immediately following the hemorrhage the blood pressure and heart rate were determined and electrical stimulation of the sciatic nerves was begun using a Harvard inductorium. Four volts were applied to the primary coil from a storage battery, and the secondary coil was set at 6 to 7 cm. on the inductorium scale. A few preparations were stimulated by a thyratron controlled discharge of 0.5 mfd. condenser so adjusted as to deliver 30 volts at a frequency of 30 to 40 c.p.s.

Throughout the succeeding 6 hour period, during which the animal remained on the board, blood pressures and heart rates were recorded every 20 minutes, rectal temperatures were measured every hour and blood samples for hematocrit values were taken occasionally. One to 2 hours after hemorrhage and application of the stimulus, a second blood volume determination was made. Stimulation of the central ends of the sciatic nerves was continued throughout the 6 hour period.

When the animal survived beyond the sixth hour, the wounds were closed, and the dog put into a cage for observation the following day. No water was given overnight. Animals which were alive 24 hours later were considered to have survived.

**RESULTS.** In three preliminary experiments we confirmed the earlier observation that stimulation of both sciatic nerves over a long period of time (6 hrs.) in an otherwise normal animal will not result in shock. Such animals respond to the application of the electrical stimulus by showing an immediate slight rise in mean arterial pressure and a small increase in the heart rate. The blood volume showed no appreciable change, a slight rise in red cell volume being balanced by a decrease in plasma volume. Normal dogs as well as those which had a sublethal hemorrhage showed, upon recovery from ether anesthesia, only a few minutes of restlessness—even though the stimulus was being constantly applied to the sciatic nerves. Any increase in voltage during stimulation resulted in a short period of renewed restlessness, following which the animal again became quiet.

<sup>4</sup>The total quantity of blood removed in this series of 30 dogs varied from 26 to 37 cc. per kgm. of body weight (average, 33 cc. per kgm.). This figure is considerably smaller than that in the simple hemorrhage series (from 32 to 47 cc. per kgm. with an average of 40 cc. per kgm.; see 2).

TABLE 1

Data on dogs with sublethal hemorrhage and sciatic stimulation, including changes in blood volumes, hematocrit values and serum protein concentrations

DOG NUMBER AND SEX	WEIGHT IN KGM.	CONTROL			AFTER HEMORRHAGE			CHANGE			FATE
		B. V.	Hct.	Protein	B. V.	Hct.	Protein	$\frac{(1)-(4)}{(1)}$	Hct.	Protein	
		(1)	(2)	(3)	(4)	(5)	(6)	B. V. 100X	(5)-(2)	(6)-(3)	
		cc/kg	per cent	gm per cent	cc/ kg	per cent	gm per cent	per cent	per cent	gm per cent	
S 1 ♂	11.4	119	50.9	6.0	84	46.3	5.3	29	-4.6	-0.7	Survived
S 2 ♂	9.9	98	41.1	5.4	83	46.1	5.4	15	+5.0	0.0	Survived
S 3 ♂	7.0	97	47.9	5.4	75	50.5	5.2	23	+2.6	-0.2	Survived
S 4 ♂	10.8	95	47.8	5.4	73	51.6	4.8	23	+3.8	-0.6	Survived
S 5 ♂	8.8	94	46.7	5.7	71	42.4	5.1	24	-4.3	-0.6	Survived
S 6 ♂	11.5	108	45.2	5.1	71	36.7	4.8	34	-8.5	-0.3	Survived
S 7 ♂	8.7	103	40.5	5.7	71	42.2	5.3	31	+1.7	-0.4	Died, 3.7 hr.
S 8 ♂	11.6	98	35.2	5.4	70	34.3	5.3	29	-0.9	-0.1	Survived
S 9 ♂	14.0	97	52.7	5.8	70	51.0	5.1	28	-1.7	-0.7	Died, 2.5 hr.
S 10 ♀	11.8	98	44.4	5.9	69	45.4	5.4	30	+1.0	-0.5	Died, 2.7 hr.
S 11 ♂	6.8	104	43.6	6.0	68	36.7	5.8	35	-6.9	-0.2	Survived
S 12 ♂	9.4	109	42.0	5.4	68	36.5	5.0	38	-5.5	-0.4	Died, 2.3 hr.
S 13 ♀	11.8	89	46.8	5.7	67	48.3	5.3	25	+1.5	-0.4	Survived
S 14 ♀	9.4	103	38.8	5.7	67	34.5	5.0	35	-4.3	-0.7	Survived
S 15 ♀	7.9	90	47.6	5.2	67	47.9	5.2	26	+0.3	0.0	Died, 3.8 hr.
S 16 ♀	8.3	86	34.8	5.0	66	43.2	5.0	23	+8.4	0.0	Survived
S 17 ♂	15.6	108	38.3	6.2	66	32.2	5.3	39	-6.1	-0.9	Died, 2.1 hr.
S 18 ♂	10.7	99	52.8	6.5	65	52.4	6.2	34	-0.4	-0.3	Died, 3.6 hr.
S 19 ♀	10.5	98	54.8	6.0	64	54.4	5.2	35	-0.4	-0.8	Died, 6 <sup>+</sup> hr.
S 20 ♂	8.7	87	45.3	5.8	63	46.9	5.7	28	+1.6	-0.1	Died, 4.3 hr.
S 21 ♂	9.0	90	41.8	6.8	63	42.6	6.3	30	+0.8	-0.5	Died, 3.1 hr.
S 22 ♀	7.5	96	40.3	5.9	63	33.0	5.2	34	-7.3	-0.7	Died, 4.5 hr.
S 23 ♂	6.3	101	34.5	4.7	63	33.2	4.3	38	-1.3	-0.4	Died, 1.8 hr.
S 24 ♂	7.2	84	47.6	5.2	62	44.6	4.6	26	-3.0	-0.6	Died, 6 <sup>+</sup> hr.
S 25 ♂	10.8	85	38.5	5.2	62	32.8	4.4	27	-5.7	-0.8	Died, 4.6 hr.
S 26 ♀	7.3	86	51.9	6.7	62	53.0	6.2	28	+1.1	-0.5	Died, 5.2 hr.
S 27 ♂	10.6	94	50.2	6.4	60	47.6	5.9	36	-2.6	-0.5	Survived
S 28 ♂	8.5	95	45.9	5.6	60	43.8	5.5	37	-2.1	-0.1	Survived
S 29 ♂	9.0	91	48.8	5.7	59	42.6	4.6	35	-6.2	-1.1	Died, 3.4 hr.
S 30 ♀	7.2	103	46.3	4.8	58	39.4	4.6	44	-6.9	-0.2	Died, 3.1 hr.
Mean.....	9.6	96.7	44.7	5.7		43.0	5.2		-1.7	-0.5	
Standard error .....		1.5	1.0	0.1					0.7	0.05	

In the series of 30 animals with residual blood volumes between 58 and 84 cc. per kgm. the over-all mortality was 57 per cent (table 1), whereas in the series of 24 animals subjected to a comparable simple hemorrhage with residual blood volumes between 58 and 77 cc. per kgm. (2) the over-all mortality was only 25 per cent. A further statistical analysis of the data by the method suggested by Doctor Fertig (see 2) shows that the residual blood volume at the 50 per cent mortality point ( $L. H_{50}$ ) is  $69.0 \pm 2.5$  cc. per kilogram of body weight and the percentage survival at a residual volume of 66 cc. per kgm., which is the value

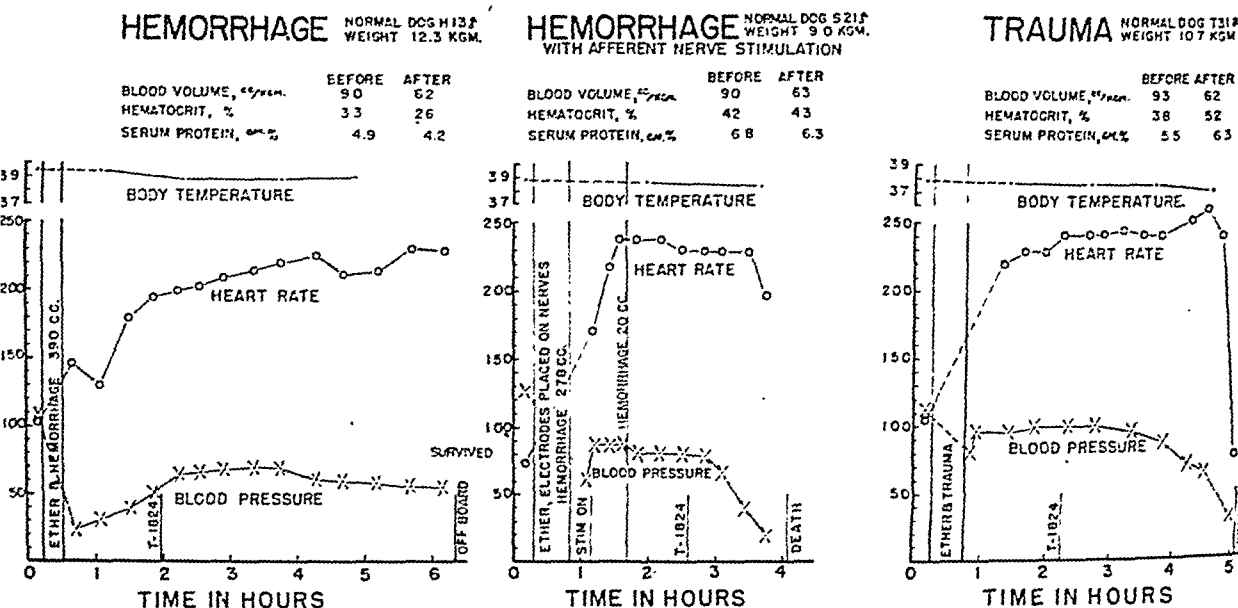


Fig. 1. Comparison of the clinical manifestations (body temperature, pulse rate and mean blood pressure) in an animal which has been subjected to hemorrhage and stimulation (S 21) with those in a typical hemorrhaged animal (H 13) and those in a typical traumatized animal (T 31). Note the similarities between dog (S 21) and dog (T 31) in the early tachycardia exceeding 200 beats per minute, high levels of mean blood pressure and death with a rapid decline of the pressure. The experimental data on dogs H 13 and T 31 are taken from a previous publication by Wang, et al (2).

previously used in comparing various series of animals (see 2), is  $37 \pm 9.5$  per cent. The average change of the hematocrit values was a decrease of  $1.7 \pm 0.7$  per cent (from 44.7 to 43.0 per cent) and that of serum protein concentrations, a decrease of  $0.5 \pm 0.05$  gram per cent (from 5.7 to 5.2 gram per cent).

In a previous report (2) we have pointed out differences in the clinical signs manifested by dogs in shock from hemorrhage on the one hand and from muscle trauma on the other. We may make a similar analysis for dogs subjected to a sublethal hemorrhage and afferent stimulation. Figure 1 illustrates in a graphic manner such a comparison of three animals with almost identical control blood volumes (90 to 93 cc. per kgm.) and residual blood volumes (62 to 63 cc. per kgm.). The dog with simple hemorrhage (H 13) was placed in a cage at the end of the sixth hour period of observation and survived. The animal (S 21) that received afferent stimulation after a sublethal hemorrhage died in about three hours. The animal (T 31) with a comparable blood volume reduction after muscle trauma died in approximately four hours.

The clinical manifestations of the three animals (fig. 1) are also typical of shock produced by the three different procedures. The initial blood pressure following the simple hemorrhage was low, whereas in the other two experiments the pressure was considerably higher. Also, the plateau blood pressure levels of the dog subjected to hemorrhage plus stimulation and the traumatized dog were similar, and both were higher than that shown by the animal in which the blood volume was reduced by simple hemorrhage. The absolute level of heart rate reached by all three animals is essentially the same. However, whereas the animal which was hemorrhaged and then stimulated, like the traumatized animal, showed heart rates exceeding 200 beats per minute immediately following the insult, the animal receiving a simple hemorrhage took some time to develop as rapid a pulse.

**DISCUSSION.** There is a considerable body of evidence purporting to prove or disprove the existence of an afferent factor in traumatic shock (7, 3). The literature dealing with the exclusion of the nervous factor by interruption of the afferent pathways will be discussed elsewhere (8). This discussion will be limited to the direct evidence of the injurious effect of afferent nervous stimulation.

Slome and O'Shaughnessy (9) made records of nerve action potentials showing an almost continuous barrage of impulses in the afferent nerves from a traumatized limb which persisted until death of the animal. This interesting observation has not been confirmed by Cressman and Benz (10), nor is it certain that the impulses are nociceptive.

Slome and O'Shaughnessy (9) have also shown that shock can be produced in the cat by appropriate trauma to a perfused hind limb separated completely from the general circulation and communicating with the body only by means of its nerves. Such a finding, though confirmed by Lorber, Kabat and Welte (11), is not substantiated by Bell, Clark and Cuthbertson (12) nor by Blalock and Cressman (13). In any case, it is difficult to interpret this type of experiment because it was done under deep anesthesia.

A number of investigators (14, 15, 16, 17) have stimulated the sciatic or other somatic afferent nerves in unsuccessful attempts to produce a depression of blood pressure and shock. Recently, Phemister and his associates (18, 19) stimulated the sciatic nerves in rabbits under urethane and in dogs under sodium barbital, and obtained only pressor responses. Our work on uncomplicated hemorrhage (2) enabled us to ascertain the ability of the animals to withstand hemorrhage and thus to carry out a series of sublethal hemorrhage experiments coupled with continuous electrical stimulation of the sciatic nerves.

In our series stimulation was started immediately after the massive hemorrhage while the animals were still under light ether anesthesia. Nevertheless, they showed certain nociceptive somatic responses. One to two minutes after the application of the stimulus the animals became quiet. The mean blood pressure showed a large increase at first which is probably related to the restlessness. Within a minute or two, the blood pressure returned to a level 25 to 35 mm. Hg above the reading taken immediately before the stimulus was applied despite the fact that the animal remained quiet during these times. The heart rates

were maintained over 200 beats per minute. These effects persisted in most instances, for if the stimulus was suddenly discontinued, both blood pressure and heart rate gradually decreased. The central stump of the sciatic nerves was still irritable at the end of the 6 hours of stimulation.

The residual blood volume at the 50 per cent mortality point in this series is  $69.0 \pm 2.5$  cc. per kgm. The difference between this and the corresponding value in the simple hemorrhage series ( $59.1 \pm 2.9$  cc. per kgm.; see 2) is statistically significant at the one per cent level. When compared with the corresponding value of the trauma series ( $73.4 \pm 3.0$  cc. per kgm.), the difference is not significant. The percentage survival at a residual volume of 66 cc. per kgm. in this series ( $37 \pm 9.5$  per cent) is also not significantly different from that in the trauma series ( $25 \pm 8.3$  per cent), but it is significantly different from that in the simple hemorrhage series ( $76 \pm 8.7$  per cent). It should be noted here that all these groups of animals had essentially the same control blood volumes on the average.

*Our data indicate clearly that strong sciatic stimulation plays an important rôle in bringing about a high mortality rate in animals with a reduced blood volume. However, in normal animals electrical stimulation of the sciatic nerves has little effect; certainly it alone will not produce shock. Indeed, we have not been able by afferent stimulation to put any dog with a residual blood volume over 75 cc. per kgm. into fatal shock.*

We are aware of the fact that electrical stimulation of the sciatic nerves may not duplicate the effect of afferent nerve impulses from an injured area. Nevertheless, it is interesting to note that the clinical signs observed on the animals in this series were like those shown by traumatized animals: early tachycardia (over 200 beats per min.), relatively high mean blood pressure, early central nervous depression and sudden death with a rapid decline of blood pressure. In the dogs which were subjected to sublethal hemorrhage plus afferent nerve stimulation, there is on the average a smaller fluid shift than in the animals which had hemorrhage alone. This is indicated by the smaller change of serum protein concentrations ( $-0.5 \pm 0.05$  per cent) and hematocrit values ( $-1.7 \pm 0.7$  per cent) as compared to the corresponding values for the hemorrhage series ( $-0.9 \pm 0.06$  per cent and  $-4.8 \pm 0.8$  per cent; see 2). The small decrease in hematocrit values after hemorrhage plus afferent stimulation may also be explained in part by an increased red cell volume produced by a greater splenic contraction than occurred after simple hemorrhage.

It may appear paradoxical that increased blood pressure following sciatic stimulation exerts a deleterious effect upon the condition of the shocked animal. Indeed, in our experience the animals that give the most marked sciatic pressor responses are those that show an early depression of the central nervous system and death. In an animal with an already reduced blood volume, the cardiac output is decreased and the tissue is anoxic. If through sciatic stimulation the vessels are further constricted, despite the resultant high blood pressure, there will be a further reduction of peripheral blood flow, tissue damage and death. The failure of previous workers to produce shock by afferent nerve

stimulation can be accounted for thus by the fact that they (a) used anesthetized animals, (b) expected an immediate depressor response following electrical stimulation of the somatic nerves and (c) did not use animals with a sublethally reduced blood volume. The last indicates clearly that afferent nerve stimulation alone will not produce shock and death.

#### SUMMARY AND CONCLUSIONS

Electrical stimulation of the sciatic nerves produces a pressor effect in sublethally hemorrhaged dogs. Nevertheless, the residual blood volume at the 50 per cent mortality point ( $69.0 \pm 2.5$  cc. per kgm.) is significantly higher than the corresponding value in the simple hemorrhage series ( $59.1 \pm 2.9$  cc. per kgm.).

Evidence is presented which indicates that the clinical manifestations of dogs subjected to sublethal hemorrhage plus afferent stimulation are modified toward those shown by the traumatized animals: early tachycardia exceeding 200 beats per minute, relatively high mean blood pressure and early central nervous depression. The changes of serum protein concentrations and hematocrit values are also less than the corresponding changes in the simple hemorrhage series.

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# RESPONSE OF THE GASTROINTESTINAL TRACT TO INGESTED CEREAL STARCH

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In connection with investigations on the response of the gastrointestinal tract to ingested carbohydrates it seemed of interest to study the response to cereal starch and to note whether the absorption of its digestion products was as closely dependent upon the emptying activity of the stomach as was the case when dextrose solutions were fed. We have previously shown that the emptying rate of the stomach varied with the size of the ingested dextrose meal and decreased with time (1, 2, 3). This is in agreement with Van Liere et al. (4) who have shown by means of fluoroscopy that increasing the size of a farina meal increased the emptying rate of the stomach. Rafferty and MacLachlan (5) showed a constant rate of absorption following the feeding of a starch suspension, in distinct contradiction to our finding with dextrose.

**EXPERIMENTAL.** The experiment was set up to simulate as nearly as possible the conditions found in man after ingestion of a cooked breakfast cereal. Young mature rats were fasted 48 hours before being fed an accurately weighed amount of a highly refined, cooked cereal. To avoid the excitement produced by handling, the animals were weighed 12 to 16 hours before the actual experiment. The cereal, weighed into a small glass dish, was slipped into the cage at the beginning of the absorption period. To facilitate the detection of any spilled cereal, glazed paper was placed under the cages. Rats failing to finish eating within 15 minutes were discarded. At the end of the absorption period the animals were killed by a blow on the head and the contents of the stomachs and intestines analyzed for reducing sugar before and after hydrolysis with 3:2 hydrochloric acid. Great care was taken to maintain a constant moisture content of the cooked cereal prepared just prior to each experiment. The cereal was fed at two levels, furnishing 172 to 173 mgm. of starch in one case and 275 to 279 mgm. in the other. The dry cereal was analyzed for starch, free sugar, protein, fat, ash, crude fiber and moisture.

**RESULTS.** The average results obtained with each of the six series of animals are shown in the table. The absorption periods with the two series were not identical because it was judged on the basis of previous work (6) that with the small meal, absorption might be complete in two hours. Interestingly enough this proved not to be true. If one plots graphically the percentages of starch hydrolyzed, emptied and absorbed against time, the respective curves for the two starch levels are almost superimposable. Some uncertainty exists concerning

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the extent of hydrolysis. The values in the table are the maximum and minimum, the former obtained on the assumption that all the reducing sugar found in the stomach was maltose, the latter on the assumption that the gastric reducing sugar was dextrose. Other work done in this laboratory has shown that the reducing sugar in the rat stomach during starch digestion was principally maltose (6). Thus the true value for the percentage hydrolysis lies probably nearer the maximum than the minimum shown in the table.

TABLE 1

*Summary of results on digestion, gastric emptying and absorption*

Starch fed—mgm.....	172	173	173	279	278	275
Time—min.....	40	80	120	60	120	180
Number of animals.....	24	24	24	26	25	7
Body weight—grams.....	143	148	146	145	135	136
Emptying—mgm.....	103	145	159	195	252	269
%.....	60	84	92	69	90	98
Hydrolysis—%						
Maximum*.....	87	94	96	83	94	97
Minimum*.....	76	89	94	77	92	97
Absorption—mgm.....	92	136	149	182	246	265
%.....	53	79	86	65	89	96

\* See text.

DISCUSSION. The observation of a higher emptying rate with the larger meal agrees well with that of Van Liere, Sleethe and Northup (4) and others. Regardless of the amount fed, the fraction emptied in any given time interval was nearly the same. This strongly suggests that an increase in the size of the meal stimulated the emptying mechanism perhaps by raising the intragastric pressure as suggested by Gianturco (7). We have shown that increasing the size of a dextrose meal increased the gastric emptying rate and that the gastric emptying rate of dextrose decreased with time (1, 2, 3). It can be concluded, therefore, that the gastrointestinal tract's response to dextrose and to cereal starch was in these respects qualitatively identical.

That the rate of absorption of the digestion products increased with increasing meal size, that the rate of absorption decreased with time and that the absorption rate paralleled the gastric emptying rate again agrees with our findings in connection with the behavior of dextrose solutions. By comparing the cereal starch data with those of the dextrose experiments with animals of colony A (2), selecting those series of comparable amounts of carbohydrate and length of experimental period, it can be seen that the emptying and absorption rates of dextrose solutions were somewhat greater than those of cereal starch. The data of Rafferty and MacLachlan (5) also showed starch to be absorbed somewhat more slowly than a corresponding amount of dextrose. It seems clear then that the necessity of digesting starch slows the absorption process to a small degree. These workers also reported a constant rate of absorption over a three-hour period after starch feeding. While this differs from our findings, their experimental conditions were



vastly different from ours. It is recognized that the amount of cereal fed in our experiments was necessarily small; yet enough carbohydrate was present to sustain a higher absorption rate than was actually observed.

#### SUMMARY

1. Gastric emptying and intestinal absorption were increased by increasing the size of the meal although the percentage emptied and absorbed was essentially unchanged.

2. The rates of emptying and absorption decreased with time.

3. The response of the gastrointestinal tract to ingested cereal starch is similar to its response to dextrose fed at similar levels.

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# SURVIVAL OF EXCITABILITY OF FROG MUSCLE, NERVE AND REFLEXES AFTER SOMATIC DEATH

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E. Mangold (1903) mentions that Du Bois-Reymond in 1859 reported survival of excitability in frog gastrocnemius for ten days, the muscle being kept at about 0°C. E. H. Brunquist (1937) found that frog sartorius muscle immersed in Ringer solution remained excitable for 8 to 142 days, average 26 days, when kept at 5 to 6°C.; 12 days at 13°C., 4.6 days at 25°C. The response was tested by direct stimulation of the muscle. I have not found analogous observations on the preservation of excitability in nerves and of reflexes. It is perhaps natural to assume that nerve fibers, nerve endings and nerve cells should be more vulnerable and liable to early death. The following experiments show that this is not the case. With the preparations kept in refrigerated dextrose Ringer solution near 0°C., the excitability of the peripheral nerves and myoneural junction, and of spinal reflexes, survived one to three weeks, almost as long as the direct excitability of muscle. All weaken progressively in similar curves; the parallelism is so close that their death must involve the same changes, including putrefaction, autolysis and coagulation of the protoplasm. The survival could be somewhat prolonged by antiseptics and rigorous control of temperature.

EXPERIMENTS ON MUSCLE AND NERVE. *Method.* Leopard frogs were decapitated, eviscerated, skinned and dissected into four preparations, two brachial and two sciatic, leaving the nerve attached to the muscle at one end, and a bit of vertebra on the other for convenience of handling. In some cases the nerve was isolated and superfluous tissue trimmed away; but in most the dissection was kept to a minimum. The results did not differ materially. The limbs of one side were generally used for these "normal" tests; those of the other side were subjected to various reagents as will be described in another paper.

The preparations were at once immersed in about 50 cc. of a refrigerated dextrose-Ringer solution (per cent: NaCl 0.65; KCl 0.015; CaCl<sub>2</sub> 0.015; NaHCO<sub>3</sub> 0.02; MgCl<sub>2</sub> 0.01; dextrose 0.1), and stored in open beakers in the refrigerator, close to 0°C., and tested from time to time, the fluid being renewed three times weekly.

To test the excitability, the preparations were removed from the cold fluid and stimulated with the faradic current of a Harvard induction coil activated by a 1½ volt dry cell; placing the platinum electrodes on the muscle, and again on the nerve at a safe distance from the muscle to guard against spread of current. The responses with the secondary coil at 12, 6 and 0 cm. were graded by an arbitrary scale and plotted against time on semilogarithmic paper, so that interpolations could be made if desired. Starting with preparations giving a

very good response at 12 cm., the reaction was timed for: (1) *Beginning of decline*, the first definite downward trend for stimulation at 12 cm. (2) *Half decline*, with slight response at 12 cm., fair at 6 cm., and good at 0 cm. (3) *Inexcitable*, with no or scarcely perceptible response at 0 cm.

The results are shown in table 1.

The decline of muscle and nerve begins at the same time, median 7 days; extreme 15 days. The further decline in muscle is 2 or 3 days slower than in nerve (very rarely faster), reading "half" in the median of  $10\frac{1}{2}$  days for nerve, 13 days for muscle; inexcitability is reached in  $12\frac{1}{2}$  days for nerve and 15 days for muscle.

TABLE 1  
*Decline of excitability of refrigerated frog muscle-nerve preparations*  
Time in days, median and (maximal)

	BEGINNING OF DECLINE	HALF DECLINE	INEXCITABLE
29 Hind legs			
Nerve trunk stimulation.....	7 (11)	10 (15)	13 (17)
Direct muscle stimulation.....	7 (11)	12 (19)	15 (29)
33 Fore legs			
Nerve trunk stimulation.....	8 (15)	11 (16)	12 (15)
Direct muscle stimulation.....	7 (11)	14 (20)	15 (22)
62 Legs, hind and fore			
Nerve trunk stimulation.....	7.5 (15)	10.5 (16)	12.5 (17)
Direct muscle stimulation.....	7 (11)	13 (20)	15 (29)

The excitability of the nerve fibers and endings, therefore, survives almost as long as that of the muscle.

*Survival of the central nervous system of eviscerated and refrigerated frogs.* This was tested by reflex movements of the opposite side, in response to faradic stimulation of the skin or of the tissues. The experiments varied between evisceration with skin left on, keeping the preparation in a moist chamber; decapitation and evisceration with skin left on, preserved in moist chamber; and decapitated, eviscerated and skinned frogs preserved in refrigerated dextrose-Ringer solution, all kept at temperatures close to 0°C.

The results were practically the same: The spinal contralateral reflexes remained normal for at least 3 to 5 days; they became significantly weaker in 11 to 14 days; greatly weakened and sluggish in 14 to 17 days; and abolished in 11 to 17 days. In these preparations, the response of the muscle to direct stimulation became significantly weaker in 14 to 17 days; greatly weakened in 17 days; abolished in 11 to 21 days. The median time of survival in these preparations was 17 days for spinal reflex, 18 days for direct nerve-muscle stimulation, 21 days for direct muscle stimulation. The spinal nerve cells, therefore, survived somatic death for two and a half weeks, practically as long as the peripheral nerve-

muscle link. In fact, the course in an eviscerated frog was practically indistinguishable from that of a normal frog kept under identical refrigeration.

Not all reflexes survive equally well. Those from the arm survived three to seven days longer than those from the leg (two experiments averaged 15 days for the leg, 20 for the arm). Reflex in response to *acid stimulation* (immersion of foot of decapitated and eviscerated frog in 5 per cent acetic acid) remained good for four hours of refrigeration but failed overnight, while reflex response to electric stimulation of the same foot remained good for ten days and fair to the twelfth day. A decapitated, eviscerated and skinned frog showed good escape reaction immediately after operation, when other spinal reflexes were poor (presumably decerebrate inhibition); but in 3 hours the escape reaction was abolished, while reflexes lasted over six days. The *embrace reflex* in a pectoral girdle preparation which was good immediately after the operation, became poor by next morning. Reflex *eye movements* on stimulation of the contralateral skin of the decapitated head remained good for 3 days in refrigerated dextrose Ringer solution; they were poor in 14 days, abolished in 17 days.

#### CONCLUSIONS

The excitability of nervous tissue, central as well as peripheral, survives somatic death almost as well as that of skeletal muscle: For frog preparations kept near 0°C., the survival time was:

	MEDIAN	MAXIMAL
	<i>days</i>	<i>days</i>
Response to direct muscle stimulation.....	15	29
Response to direct nerve stimulation.....	12½	17
Response to spinal reflex stimulation.....	17	17

Very little deterioration was demonstrated in the first half of this period, then the responses decreased markedly and progressively.

In the central nervous system some reflexes are extinguished much more promptly than others; for instance, the response of the spinal reflex to acid before that of electric stimulation.

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# HEPATIC BLOOD FLOW AND GLUCOSE OUTPUT IN NORMAL UNANESTHETIZED DOGS

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The angiostomy technique as developed by London (1) has made it possible to examine many of the metabolic activities of the liver under substantially normal conditions. However, it has not previously been possible to determine hepatic blood flow in angiostomized animals, and the addition or removal by the liver of substances present in the blood could only be given as quantity per unit volume of blood. This limitation has restricted the conclusions that could be drawn from experiments on angiostomized animals to qualitative changes.

We have now developed a method for the determination of the quantity of blood passing through the liver per unit time in the angiostomized dog. As will be shown, this method has its own limitations. It is not adapted to the measurement of rapid changes in blood flow, but can determine only the average rate of flow over a period of time. However, such determinations of mean rate should be entirely satisfactory for steady states in fasting animals. In order to establish the validity of this method, to make comparisons between it and other methods for blood flow, and to compare the calculated glucose output of the liver with outputs as determined by other techniques, we have carried out simultaneous blood flow determinations, and measurements of glucose concentration in the inflowing and outflowing liver blood, in normal fasting dogs.

**METHODS.** The technique of applying London cannulae has been described previously (2). For the last two years we have been using Vitallium cannulae<sup>2</sup> which we prefer for their greater resistance to wear and because they seem to become more promptly and rigidly fixed to the veins.

Our method for the determination of hepatic blood flow depends upon two premises: 1, that all of the urea appearing in the urine has its origin in the liver, and 2, that all of the urea liberated by the liver appears as such in the urine. The first of these has been generally accepted since the experiments of Bollman, Mann, and Magath (3). The recent demonstration by Van Slyke et al. (4) that the ammonia of the urine is derived from glutamine and amino acids and not from urea appears to leave no possible fate for urea other than quantitative excretion in the urine. If, then, the quantity of urea N (in mgm.) appearing in the urine during a given period of time is divided by the increase in urea N concentration (in mgm. per liter) in the blood due to its passage through the liver, and the resulting value is corrected for any changes in blood urea N concentration, we obtain the quantity of blood flowing through the liver during that

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period. The amount of urea N added by the liver to each liter of blood has been found to be reasonably constant over a period of several hours in the fasting dog, as is the urine urea N output. The concentration of urea N in samples of portal blood and of arterial blood has been found to be the same, within the limits of error of our method for urea N determination, in well over 100 consecutive analyses. The increase in urea N concentration which is due to the addition of urea by the liver may therefore be determined by subtracting either the portal or arterial value for BUN from the BUN of the hepatic venous blood.

As finally systematized, our procedure is as follows: a fasting London cannula dog, which has been handled often enough so that it has lost any apprehensiveness, is catheterized, all urine is removed, and the bladder is washed out twice with measured amounts of water. Blood samples are then taken from the hepatic and portal veins and from the femoral artery, and the animal is returned to its cage. At the end of exactly two hours, and again exactly four hours after the first catheterization, the bladder is again catheterized and washed and blood samples are taken. The urine samples and washings from the two and four hour catheterizations (but not from the zero hour catheterization) are saved, and urea N determinations by Folin's method (5) are made on each sample after combining it with its washings. BUN determinations on the arterial or portal venous and on the hepatic venous blood samples are then made. In order to determine the blood flow in liters per kilogram of body weight per hour, the total urine urea N output for the four hour period is divided by four, and this figure is divided by the weight of the dog in kilograms. A correction for retention or loss of urea in or from the body fluids is then applied; this is usually very small. Since urea is freely diffusible across cell boundaries we assume that it is distributed throughout  $\frac{2}{3}$  of the body weight. If the portal venous (or arterial) BUN has changed during the four hour period, the difference between the first and last reading in milligrams per liter is obtained, and this figure is divided by four to get the average change per hour. The result is multiplied by  $\frac{2}{3}$  to obtain the retention per kilo of dog on the assumption of distribution of urea through  $\frac{2}{3}$  of the body weight. This figure is then added to the per kilo per hour urine urea N output if urea was retained, i.e., if the BUN rose, or subtracted from it, if urea was lost (if the BUN fell). The corrected value for urine urea N is divided by the average amount of urea N added to each liter of blood by the liver, as obtained from the BUN determination on the three sets of blood samples, which gives the hepatic blood flow in liters/kilo/hour.

Because the increase in BUN on the passage of blood through the liver is usually somewhat less than 1.0 mgm., the method employed to determine the BUN must be more accurate than those in common use. We have found the method of Conway (6), with some modifications, to be satisfactory; its accuracy in our hands, has been  $\pm 0.1$  mgm. urea N/100 ml. When dealing with such small differences, chance contamination may introduce serious errors and is difficult to avoid entirely; we have therefore routinely determined the hepatic

venous BUN in triplicate, and have made duplicate determinations on both portal venous and arterial blood samples, although as pointed out above triplicate determinations on either the portal venous or arterial sample would be adequate.

Since the zinc filtrates used for glucose determinations form a surface scum on the addition of potassium carbonate in the Conway method for urea, the determinations of BUN were made on tungstate filtrates.

Two milliliters of 1:10 filtrate were placed in the outer ring of the Conway unit, and 2 ml. of 0.05 N  $\text{H}_2\text{SO}_4$  in the inner ring. To the filtrate were then added 0.2 ml. of a 20 per cent solution of Squibb's urease and one drop of phosphate buffer. The covers were then placed on the units, the seal being made with alkaline tragacanth. The units were then placed on a motor driven rocking board and rocked gently for 20 minutes, after which 1 ml. of saturated  $\text{K}_2\text{CO}_3$  was added to the filtrate. The rocking was then resumed, and after 2 hours the  $\text{H}_2\text{SO}_4$  solution of the inner ring was transferred by suction to a glass stoppered test-tube graduated at 10 ml. The inner ring of the unit washed with 4 successive 1.5 ml. quantities of water, each washing being sucked into the test-tube. The contents of the test-tube were then made up to 10 ml., 1.0 ml. of Nessler's solution added, and the colors were read photoelectrically (Cenco Photometer, blue filter). It is hardly necessary to point out that the most scrupulous care is necessary with regard to cleanliness and completeness of transfer if the required accuracy is to be obtained.

In the determinations of glucose output by the liver, the blood glucose in the hepatic and portal venous and the arterial samples was determined by the method of Shaffer and Somogyi (7). The average glucose concentration of the inflowing blood was determined on the assumption that the portal vein contributed  $\frac{3}{4}$  and the hepatic artery  $\frac{1}{4}$  of the blood reaching the liver. As a rule, the amount of glucose removed in the intestinal tract is too small to introduce any appreciable error even should these proportions change materially. The glucose determinations were made on all three sets of blood samples, and the average concentration of glucose in milligrams per liter added by the liver was determined. Multiplying by the hepatic blood flow, the quantity of glucose which the liver adds to the blood per hour may then be calculated.

**RESULTS.** Complete data on two typical experiments are presented in tables 1 and 2; in the first, urine and blood samples were collected every hour for four hours while in the second, the collections were at intervals of two hours over a four hour period. The results of 20 such experiments carried out on eight normal fasting dogs are presented in table 3. It was observed in preliminary experiments that unless the starvation period was of one of two days' duration, the output of urea N by the liver might be too small to permit satisfactory measurement. However, after at least two days without food, the average BUN difference between inflowing and outflowing blood was less than 0.6 mgm./100 ml. in only three experiments, and was frequently as high as 1.0 mgm. The standard errors for blood flow and glucose output indicate that one would expect the average value for blood flow to vary from 1.5 to 2.3 liters/kilo/hour in other

similar groups of normal animals, and the average value for glucose output in normal dogs might range from 85 to 159 mgm./kilo/hour. As will be shown below, these figures are entirely consistent with those which have been obtained by other methods.

TABLE 1

*Determination of hepatic blood flow and glucose output in dog 6*

In this experiment urine collections were made and blood samples taken every hour for four hours. Weight of dog 15.7 kgm.

TIME A.M.	URINE		BLOOD					
	Volume	Urea N	Urea N (mgm./100 ml.)			Glucose (mgm./100 ml.)		
			Portal	Hepatic	Arterial	Portal	Hepatic	Arterial
	<i>ml.</i>	<i>mgm.</i>						
8:00	Washed bladder							
9:00	34	230	15.9	16.5	15.9	76	84	78
10:00	31	221	16.0	16.5	15.9	77	83	77
11:00	31	223	16.3	17.0	16.4	79	86	84
12:00	32	225	16.9	17.5	16.9	77	83	80

*Hepatic outputs of urea N and glucose in mgm./liter of blood*

TIME (A.M.)	UREA N	GLUCOSE	AVERAGE OUTPUT UREA N IN URINE, PER KILO PER HOUR
			<i>mgm.</i>
9:00	6	70	14.4
10:00	5	60	
11:00	7	60	
12:00	6	50	
Average output per hour.....	6	60	

Increase in BUN over 4 hour period..... 10 mgm./liter

Calculated urea N retained per kilogram body weight..... 6.7 mgm.

Calculated urea N retained per kilogram of dog per hour..... 1.68 mgm.

Total output of urea N from liver (urinary output plus amount retained) per kilo per hour..... 16.1 mgm.

Hepatic blood flow (liters per kilo per hour) =

$$\frac{\text{hepatic urea N output per kilo per hour in mgm.}}{\text{hepatic urea output in mgm. per liter}} = \frac{16.1}{6} \dots\dots 2.7$$

Hepatic glucose output per kilo per hour..... 162 mgm.

DISCUSSION. A comparison of our values for hepatic blood flow with those that have been obtained by other workers is presented in table 4. Not only is our average value in good agreement with those previously reported, but the range of values obtained by our method is comparable to the variations found by others. All of the methods previously employed have involved anesthesia or procedures which might conceivably produce some obstruction to blood flow. The ten-



dency of our values to be somewhat higher than those obtained by other investigators, while not significant statistically, may perhaps be due to the fact that our animals were unanesthetized, and that, to the best of our knowledge, no venous obstruction is produced by the presence of the London cannulae. The fact that our values for blood flow agree so well with those previously reported indicates that starvation for periods up to and including 5 days has little if any effect on the quantity of blood passing through the liver.

TABLE 2

*Determination of hepatic blood flow and glucose output in dog 5*

Urine collections made over 2 four hour periods; 3 sets of blood samples taken

TIME (A.M.)	URINE		BLOOD					
	Volume	Urea N	Urea N (mgm./100 ml.)			Glucose (mgm./100 ml.)		
			Portal	Hepatic	Arterial	Portal	Hepatic	Arterial
	ml.	mgm.						
10:00	Washed bladder		12.9	13.4	12.9	71	76	70
12:00	44	165	12.9	13.3	12.9	73	78	74
2:00	40	163	12.5	12.9	12.5	73	77	74

*Hepatic outputs of urea N and glucose in mgm./liter*

TIME (A.M.)	UREA N	GLUCOSE	AVERAGE OUTPUT UREA N IN URINE PER KILO PER HOUR
			mgm.
10:00	5	50	5.73
12:00	4	50	
2:00	4	40	
Average output per hour.....	4.3	47	

Increase in BUN over 4 hour period..... 4 mgm./liter

Calculated urea N lost from body fluids per kilo per hour..... 0.7

Total output of urea N from liver (urine output minus that lost from body fluids) per kilo per hour..... 5.0 mgm.

Hepatic blood flow (liters per kilo body weight per hour) =

$$\frac{\text{hepatic urea N output per kilo per hour}}{\text{hepatic urea N output in mgm./liter}} = \frac{5.0}{4.3} = 1.2$$

Hepatic glucose output per kilo per hour..... 56 mgm.

Since the range of values obtained by us is little greater than that found by the thermostromuhr method, or by direct measurement of the blood flow from the hepatic vein, it would seem that our method is of approximately the same order of accuracy. We believe our greatest source of error to lie in the measurement of the difference in urea N concentration between inflowing and outflowing blood. If the difference is as small as 0.5 mgm./100 ml., the inaccuracy may be as great as 20 per cent since we cannot measure the concentration differences more closely than  $\pm 0.1$  mgm./100 ml. In most experiments the difference in con-

centration has been in the neighborhood of 1.0 mgm./100 ml., and the error will therefore be approximately 10 per cent. The quantity of urea eliminated in the urine can obviously be measured with much greater accuracy than the blood concentration difference. The total correction for urea N retention in or loss from the body fluids is rarely as high as 15 per cent, and even a failure of urea N retained or lost to be evenly distributed throughout the body water would presumably amount to an error of only a few per cent. Now that it has been shown (4) that the urine ammonia is not formed from urea, the assumption that all

TABLE 3

*Hepatic blood flow and hepatic glucose output in normal dogs per kilogram of body weight per hour*

EXPT.	DOG NO.	STARVED	WEIGHT	HEPATIC BLOOD FLOW	HEPATIC GLUCOSE OUTPUT
		<i>hrs.</i>	<i>kilos</i>	<i>liters/kilo/hour</i>	<i>mgm./kilo/hour</i>
1	1	47	13.2	1.1	not done
2	1	71	12.9	1.6	82
3	2	44	10.0	2.1	126
4	3	67	10.5	2.5	113
5	4	48	7.7	2.5	231
6	5	68	12.0	2.1	114
7	5	72	15.5	1.1	57
8	5	72	14.3	1.2	56
9	6	40	15.7	2.7	162
10	6	120	15.0	1.6	91
11	6	72	13.6	2.3	179
12	7	72	11.8	1.3	105
13	7	120	11.6	1.6	109
14	7	120	10.2	2.6	61
15	7	120	10.3	1.4	78
16	7	72	11.8	1.7	155
17	7	120	10.6	2.9	204
18	7	120	11.4	2.5	208
19	8	72	13.6	1.6	73
20	8	120	12.4	2.2	110
Mean.....				1.93	121.8
S.E. of mean.....				±0.13	±12.2

urea liberated by the liver appears in the urine seems to be completely justified. We therefore believe that other errors are small in comparison with that involved in the determination of blood concentration difference, and that the total error should not exceed 20 per cent and should become less than this when concentration differences higher than 0.5 mgm./100 ml. are encountered.

To the best of our knowledge, this is the first time that direct determinations of hepatic glucose output have been made on an extensive series of normal animals. Soskin et al. (8) have carried out experiments on dogs under anesthesia, measuring blood flow by the thermostromuhr and glucose concentration

difference by removal of samples from portal vein, carotid artery, and hepatic vein. Data from which glucose output in terms of milligrams per kilo per hour can be calculated are given for only one dog; in this animal during the control period (prior to glucose injection) the liver at one time removed glucose at the rate of 50 mgm./kilo/hour, and the maximum rate of addition of glucose to the blood was 160 mgm./kilo/hour. In many hundreds of blood samples taken from fasting angiotomized dogs in the course of this and previous experiments, we have never observed such a change from output to retention and back to output again within a short period of time, and we question whether it occurs

TABLE 4  
*Collected data on hepatic blood flow*

AUTHOR	DATE	NO. OF EXPERIMENTS	METHOD	AVERAGE BLOOD FLOW <sup>*</sup>	MAXIMUM AND MINIMUM VALUES
				<i>liters/kilo/hour</i>	
Burton Opitz (11).....	1911	15	Stromuhr	1.5	0.8-2.7
MacLeod and Pearce (12) ..	1914	8	Collection	2.6	1.5-3.2
Grab, Janssen, and Rein (13).....	1929	5	Thermostromuhr	1.6*	1.3-2.6
Grab, Janssen, and Rein (14).....	1929	6	Thermostromuhr	1.5*	1.1-2.0
Herrick, Mann, Essex and Baldes (15).....	1934	1	Thermostromuhr	3.7	3.3-4.3
Blalock and Mason (16)....	1936	24	Collection	1.6	1.0-2.4
Soskin, Essex, Herrick and Mann (10).....	1938	1	Thermostromuhr	2.2	0.9-3.5
Grindlay, Herrick, and Mann (17).....	1941	8	Thermostromuhr	1.5*	1.1-2.3
Grodins, Osborne, Ivy, and Goldman (18).....	1941	†	Thermostromuhr	1.4 1.7†	
Our data.....	1945	20	Urea output	1.9 ± 0.13	1.1-2.9

\* Average of inflowing and outflowing values.

† Average of 21 determinations of flow in hepatic artery plus average of 5 determinations of flow in portal vein; considered as 5 experiments for purpose of calculating weighted average.

‡ Weighted average.

under normal conditions unless the animal is fed. It seems quite possible that the extensive surgical procedures used by Soskin et al. in preparation for the placing of the thermostromuhr and collection of blood samples in acute experiments altered the behavior of the liver.

In the absence of other direct determinations of hepatic glucose output, we have compared our data with values obtained by indirect methods. Estimates of the quantity of glucose added to the blood from the liver may be obtained from: 1, determinations of the quantity of glucose required to maintain normal blood sugar levels in hepatectomized animals; 2, the glucose metabolism of the

body from determinations of gaseous metabolism and 3, the glucose appearing in the urine in pancreatic or phlorizin diabetes. Each of these methods is open to objection. We do not know the effect of hepatectomy on the metabolism of the remaining tissues; calculation of glucose being oxidized on the basis of  $O_2$  consumption and nonprotein RQ includes the glucose, if any, being oxidized by the liver; and there is at present a considerable controversy over how much glucose may or may not be oxidized in pancreatic or even phlorizin diabetes. However, it is of interest to make the comparisons, and the pertinent data from

TABLE 5

*Glucose utilization by the dog, as determined by various indirect methods*

AUTHOR	METHOD	GLUCOSE UTILIZATION OR LOSS IN URINE* <i>mgm./kilo/hour</i>
Bollman and Mann 1936 (19).....	Hepatectomy	125-250
Ibid (19).....	Same, after glucose deprivation	100
Yater, Markowitz, and Cahoon 1933 (20) .....	Hepatectomy	190
Drury, Bergman, and Greeley 1936 (21).....	Hepatectomy after phlorizin and fasting	75
Lusk 1915 (22)	Gaseous metabolism after 20 to 72 hour fast	78
Chambers and Lusk 1930 (23).....	Gaseous metabolism, basal state	213
Dann, Chambers, and Lusk 1931 (24)..	Gaseous metabolism, basal state	235
Atkinson, Rapport, and Lusk 1922 (25)..	Gaseous metabolism, basal state	249
MacLeod and Markowitz 1926 (26) ....	Pancreatic diabetes	51-187
Von Falkenhausen 1925 (27).....	Pancreatic diabetes	123-181
Chambers and Coryllos 1926 (28).....	Pancreatic diabetes	61-126
Chambers, Himwich, and Kennard 1935 (29).....	Pancreatic diabetes	65-149
Wierzuchowski 1926 (30).....	Phlorizin diabetes	111-120
Csonka 1915 (31).....	Phlorizin diabetes	98-152
Ringer and Frankel 1914 (32).....	Phlorizin diabetes	67-118
Ringer 1912 (33).....	Phlorizin diabetes	115-208
Nash 1923, 1925 (34) (35).....	Phlorizin diabetes	84-152

\* Range is given where stated by authors.

various sources is collected in table 5, after the results of the various authors have been translated into terms of glucose per kilogram of body weight per hour. It is perhaps significant that the glucose requirement of the hepatectomized dog, the rate of glucose oxidation as determined by measurements of gaseous metabolism, and the glucose lost in the urine in phlorizin or pancreatic diabetes all fall within the same range (60 to 250 mgm. per kgm. per hour). There is general agreement that glucose utilization by fasting animals proceeds at a slower rate than in dogs that have been recently fed. As shown in table 3, the values for hepatic glucose output as determined by us tend to group themselves in the lower

portion of this range, the average being 121.8 mgm./kilo/hour with no values higher than 231.

Since urinary glucose output in both pancreatic and phlorizin diabetes is in the same range as the glucose requirement of the hepatectomized dog, the rate of glucose oxidation as calculated from determinations of gaseous metabolism, and the hepatic glucose output as determined by our method, the view that the metabolic fault in diabetes is inability to utilize glucose rather than an overproduction of this sugar by the liver is strengthened. One would scarcely expect overproduction to cause the appearance in the urine of an amount of glucose equivalent to that which the animal normally oxidizes.

#### SUMMARY

A method is described by which the hepatic blood flow can be determined in normal, unanesthetized dogs with London cannulae on the portal and hepatic veins. This method depends on simultaneous determinations of urea N output in the urine and the amount of urea N added by the liver to each liter of blood.

The average hepatic blood flow in fasting dogs, so determined, is 1.9 liters/kilo/hour. Determinations of portal, arterial and hepatic blood glucose concentrations at the same time permit the calculation of hepatic glucose output, which was found to average 122 mgm./kilo/hour.

These values for hepatic blood flow are in the same range as those obtained by other methods.

Our calculations of hepatic glucose output have been compared with the glucose requirement of hepatectomized dogs, the rate of glucose oxidation as calculated from the gaseous metabolism, and the rate of glucose loss in the urine in pancreatic and phlorizin diabetes. All of these values fall within the same range, which suggests that they all may be approximate measurements of the normal rate of glucose utilization.

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# A DIRECT MEASUREMENT OF HEPATIC GLUCOSE PRODUCTION IN EXPERIMENTAL DIABETES MELLITUS

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Each of the two rival theories which seek to explain the metabolic defect in diabetes mellitus still has its proponents, and most current texts still present both views. Until the present time no direct determinations of the amount of glucose produced in the body of the unanesthetized experimentally diabetic animal have been made, and each school of thought has supported its view by indirect evidence. Those who conclude that the ability to utilize glucose is lost or depressed in diabetes point to the fact that every substance known to be glucogenic appears almost quantitatively in the urine in the form of glucose when fed to diabetic dogs, the low respiratory quotient, the absence of arterio-venous glucose differences in the extremities, and an array of other evidence indicating that little if any glucose is oxidized. The other group, favoring the view that the hyperglycemia and glycosuria are due to overproduction, have sought to show that the tissues of the diabetic animal have not lost their power to oxidize glucose, and in spite of the difficulty of demonstrating any transformation of fat to carbohydrate in the animal body, generally hold that in diabetes the excess glucose arises from fatty acids. Especially in recent years, a compromise view has gained favor, namely, that both underutilization and overproduction are present. The literature dealing with this controversy is so vast that reference can be made only to reviews in which the opposing concepts are discussed (1, 2, 3).

The development of a method for determining hepatic blood flow in intact, unanesthetized dogs (4) has made it possible to obtain direct measurements of the amount of glucose formed by the liver. Some years ago one of us (LAC) succeeded in placing London cannulae on the portal and hepatic veins of a depancreatized dog, and found that the increase in glucose concentration of the blood passing through the liver was comparable to that in normal animals, but because of the realization that such evidence would be unacceptable in the absence of blood flow determinations, further studies were deferred until simultaneous determinations of blood flow would permit calculation of the amount of glucose liberated by the liver in terms of milligrams per kilogram of body weight per hour as well as in milligrams per liter of blood. Since this can now be done, we have proceeded to study the hepatic glucose output of the diabetic dog.

**METHODS.** Except for the removal of the pancreas, we have proceeded as in previous experiments on the hepatic glucose output of the normal dog (4). Various procedures for obtaining a depancreatized animal with London can-

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nulae in place were attempted. If either the pancreas is removed before placement of the cannulae, or the cannulae are attached to the veins before removal of the pancreas, the connective tissue reaction which follows the first operation makes the second extremely difficult. Placing the cannulae at the same time the pancreas was removed was not at first successful, for healing was poor and the cannulae tended to pull loose. However, by employing Vitallium cannulae and giving the animals 2 grams of sulfathiazole per day in divided doses it was possible to obtain a high percentage of successes. Our best results were obtained by carrying out the usual preliminary operation of scarifying the portal and hepatic veins, and sewing to them patches of omentum. Then 2 to 3 weeks after this first operation the pancreas was removed, taking care to pick off all remnants of pancreatic tissue, and the cannulae were sewed to the patches on the veins and brought out through individual stab wounds (or the hepatic vein cannula may be brought out through the upper end of the right rectus incision). Sulfanilamide or sulfathiazole, one gram twice daily, was then given until healing was complete (usually 10 days). As in all experiments on angiotomized animals, the dogs were not used until 2 to 3 weeks after the second operation, at which time small samples of blood were withdrawn at frequent intervals until the animals were accustomed to the procedure and would lie quietly on the table.

The immediate postoperative care of the depancreatized dogs included the feeding of milk and the administration of small doses of insulin. Within a few days they were placed on a diet of fresh beef heart, raw pancreas, Karo syrup, and Purina checkers. Daily determinations of urine sugar were made, and the insulin so regulated that only 1 or 2 grams of glucose per day appeared in the urine; as a rule, separate injections of protamine-zinc and regular insulin were made once daily. When the animals seemed vigorous and well and were stable as to urine glucose excretion, both food and insulin were withdrawn and determinations of hepatic blood flow and hepatic glucose output were made at intervals up to the 7th day of fasting and insulin deprivation.

During the experimentation period, the urine glucose determinations were made by the method of Shaffer and Somogyi (5). All other techniques and chemical methods were the same as those previously used to determine the rate of glucose production by the liver in normal dogs (4).

**RESULTS.** The data from 15 determinations of blood flow and glucose output in the diabetic dogs are presented in table 1. Observations were made at various intervals following the withdrawal of food and insulin, so that we might observe the development of the diabetic state. A distinction must be made between the period during which the diabetic state is developing as the amount of insulin decreases, and the later stable state attained after the depots of insulin have been exhausted. Since the action of protamine zinc insulin persists for about 42 hours (6), we have averaged separately the observations made during the first 2 days after withdrawal of food and insulin, and those made 3 or more days after withdrawal. The hepatic blood flow of the diabetic dog 3 or more days after withdrawal is, on the average, slightly greater than that of the normal animal when the data from table 1 are compared with those previously presented for



normal dogs (4), but the difference is not statistically significant. In only 3 experiments was the blood flow for diabetic dogs greater than the largest blood flow in normal animals; if these 3 values are omitted the average becomes 2.04 liters/kilo/hour which is very close to the average figure of 1.93 liters/kilo/hour for normal dogs. Of the 2 highest values (first day in first series of dog 1, and

TABLE 1

*Hepatic blood flow, glucose output, and blood sugar level in depancreatized dogs at varying intervals after withdrawal of food and insulin*

DOG NO.	DAYS AFTER WITHDRAWAL OF FOOD AND INSULIN	HEPATIC BLOOD FLOW	HEPATIC GLUCOSE OUTPUT		BLOOD SUGAR LEVEL
		liters/kilo/hour	mgm./100 ml. blood	mgm./kilo/hour	
1	1	2.1	8.5	179	150
	3	3.3	8.0	264	320
	4	2.4	7.3	175	302
2	1	6.6	4.3	283	227
	3	2.9	3.7	107	345
	5	1.9	5.7	108	346
	7	1.6	7.0	112	370
2*	3	2.6	4.3	112	325
3	2	1.5	9.7	146	320
	4	1.6	4.7	75	340
	6	1.2	7.7	93	400
4	3	2.2	5.0	110	250
	5	2.4	6.3	151	425
4†	2	2.1	11.7	246	340
	4	5.1	4.0	204	380
Averages and standard errors.....		2.6 ± 0.37		158 ± 16.9	
Averages and standard errors for values obtained 3 or more days after withdrawal of food and insulin.....		2.5 ± 0.32		137 ± 17.0	

\* 30 days later.

† 17 days later.

fourth day in second series of dog 4) of 6.6 and 5.1 liters/kilo/hour, the urine urea was extraordinarily high in the first and the difference in urea concentration between inflowing and outflowing blood was unusually low in the second. In both of these experiments the calculation of glucose output in terms of milligrams per kilo per hour gives reasonable figures, which makes it appear probable that the high blood flow estimations are not the result of technical errors.

The liberation of glucose by the liver is with few exceptions within the range previously observed in normal dogs, whether expressed in terms of milligrams of glucose added to each 100 ml. of blood or in terms of glucose liberated per kilo per hour. The greatest hepatic glucose output in any normal animal was 231 mgm./kilo/hour (4), which was exceeded in only 3 out of the 15 experiments on diabetic dogs and in none of the experiments made after more than 3 days without

TABLE 2  
*Hepatic glucose output and urine glucose output in diabetic dogs*

DOG NO.	DAYS AFTER WITH-DRAWAL OF FOOD AND INSULIN	HEPATIC GLUCOSE OUTPUT	URINE GLUCOSE OUTPUT	AMOUNT GLUCOSE RETAINED BY BODY
		<i>mgm./kilo/hour</i>	<i>mgm./kilo/hour</i>	<i>mgm./kilo/hour</i>
1	1	179	2	177
	3	264	101	163
	4	175	112	63
2	1	283	15	268
	3	107	76	31
	5	108	69	39
	7	112	67	45
2*	3	112	64	48
3	2	146	66	80
	4	75	82	-7
	6	93	95	-2
4	3	110	114	-4
	5	151	128	23
4†	2	246	236	10
	4	204	113	91
Averages and standard errors for values obtained 3 or more days after withdrawal of food and insulin. ....		137 ±17.0	92.8 ±6.6	44.5 ±13.5

\* 30 days later.

† 17 days later.

food and insulin. The average value for hepatic glucose output in 19 experiments on normal dogs was  $122 \pm 12.2$  mgm./kilo which is not significantly different, statistically, from the average for all diabetic dogs of  $158 \pm 16.9$ , and is even closer to the average value for diabetic animals that have gone 3 or more days without food or insulin ( $137 \pm 17.0$ ).

In table 2 the amounts of glucose liberated by the liver in the diabetic animal are compared with the glucose appearing in the urine, the urine samples being those on which urea N output was also determined for calculation of blood flow, and

having been obtained by catheterization. The difference between hepatic glucose output and glucose lost in urine averages  $44.5 \pm 13.5$  mgm./kilo/hour, and is obviously the amount of glucose retained and presumably oxidized. The large variations in the proportion of the hepatic output that appears in the urine are not surprising, since no attempt was made to control rate of urine formation. The significance of the average figure for glucose retention by the body is revealed, however, by statistical analysis and comparison to the average hepatic glucose output in normal dogs where retention is of course equal to hepatic output. The difference between the retention in diabetic and normal dogs is  $77.3 \pm 18.2$  mgm./kilo/hour, giving a  $t$  of 4.2, which would occur by chance less than 1 time in a thousand. It is evident that definitely less of the glucose formed by the liver is utilized by the diabetic than by the normal dog.

There is some tendency for the hepatic glucose output to be higher during the first 2 days after withdrawal of food and insulin, and to fall thereafter. This is especially well illustrated by the first experiment on dog 2, tables 1 and 2. However, the difference between the average hepatic glucose output on the first and second days after withdrawal (214 mgm./kilo/hour) and the average output on and after the third day (137 mgm./kilo/hour) is only slightly more than twice its standard error ( $t = 2.2$ ,  $P = 0.05$ ), and therefore is only probably significant. The data may be regarded as suggesting, but not demonstrating, the existence of overproduction during the development of the diabetic state.

**Discussion.** It is possible that data from a larger series might validate the 12 per cent greater glucose production by the liver of the diabetic (3 or more days without food and insulin) dog as compared with the normal, but calculation indicates that the number of animals required to settle this point would be prohibitive. Furthermore, statistical analysis shows that the greatest overproduction which may conceivably occur (assuming the average hepatic glucose output to be too low by 3 times its standard error) would still not account for the glucose lost in the urine. Even on the basis of this most unlikely assumption, therefore, overproduction by the liver can not be responsible for the phenomena of the diabetic state. It is probable that overproduction by the liver is not even a significant factor.

Only the possibility that there is an appreciable extrahepatic production of glucose in the diabetic dog prevents us from coming to the definite conclusion that there is a serious impairment of glucose utilization in this animal. We are the more inclined to consider such a possibility because the glucose remaining after subtraction of the urine loss from the hepatic output is not sufficient to meet the probable requirement of those tissues known to utilize glucose in the absence of insulin. Since the demonstration by Himwich and Nahum (7, 8) that its R.Q. is nearly unity in both normal and diabetic dogs, many further observations have confirmed the almost exclusive utilization of glucose by brain under a variety of conditions. The glucose requirement of the brain may be estimated at about 50 mgm./kilo/hour. We have shown that the gastrointestinal tract continues to remove glucose from the blood in these diabetic dogs (9), and have estimated the rate of removal at 48 mgm./kilo/hour. If these

estimates are reasonably accurate, the fraction of the hepatic production that remains in the body is not adequate for the needs of these tissues. Roberts and Samuels (10) have shown that the kidney of the fasting, but not the fed, rat adds glucose to the blood. Bergman and Drury (11) have found that the amount of glucose needed to maintain the blood sugar level of the eviscerated nephrectomized rabbit is higher (an average value for the increase can not be deduced from their report) than that of the animal that is only eviscerated. They attribute the increase in the nephrectomized animal to retention of some substance normally excreted by the kidney and which when "dammed back in the body causes a marked increase in glucose requirement". It seems equally possible that the increase is due to removal of an organ which previously added glucose to the blood. In the absence of more complete information concerning extraphepatic glucose production it is patently impossible to draw up a balance sheet of glucose production and utilization. The possibility that the kidney as well as the liver of the diabetic dog carries out the process of gluconeogenesis must be considered.

However, it seems unlikely that any extrahepatic tissue could supplement the hepatic glucose output by a quantity sufficient to constitute overproduction or even to supply the skeletal muscles with any appreciable fraction of their metabolic requirement. In this connection, the reports that in human and experimental diabetes the arterio-venous glucose differences are reduced or absent even after glucose administration (12, 13, 14, 15, 16, 17, 18, 19) are significant. The absence of any appreciable difference has been observed consistently by all those who have studied severely diabetic patients or animals, but has not received the attention it deserves. Cavett and Seljiskog (20) are the only authors who find A-V differences in diabetics comparable to those of normal individuals, though the incompleteness of the diabetic state in their patients is indicated by the fact that the blood sugar was higher than 180 mgm./100 ml. in only 1 instance, and their results are therefore of questionable value.

The suggestion inherent in our data that overproduction may be occurring during the development of the diabetic state requires little comment. It seems reasonable to suppose that as the available insulin supply is decreasing and the blood sugar level rising the liver temporarily increases its output, but further evidence will be needed to establish the existence of this phenomenon.

The fact that 32 per cent of the glucose produced by the liver is not excreted in the urine obviously provides a further basis for criticism of the classical interpretation of the G:N ratio. If corrected for the glucose utilized by the body, the ratio of 2.8, which some have regarded as typical of the depancreatized dog, becomes 4.0. However, in view of the demonstrated utilization of glucose by brain and gastrointestinal tract in diabetes, and the present evidence that the fraction of glucose retained is appreciable, the ratio of glucose to nitrogen in the urine appears to be meaningless.

#### SUMMARY

1. Hepatic blood flow and glucose output have been determined at intervals after withdrawal of food and insulin from depancreatized dogs. The data are compared with previous observations on normal animals.

2. The average hepatic glucose output of the diabetic dog is 12 per cent higher than that of the normal; this difference is not statistically significant.

3. The proportion of the hepatic glucose output retained by the diabetic dog (hepatic output minus urine glucose) is 36 per cent of the hepatic output of normal dogs and 32 per cent of that of the diabetic animal itself.

4. The retained fraction of the hepatic glucose output does not appear to be adequate to supply the glucose used by brain and gastrointestinal tract, both of which continue to remove glucose in the diabetic state. Extrahepatic glucose production, possibly by the kidney, is suggested.

5. The fact that 32 per cent of the glucose produced by the liver is utilized by the body invalidates the classical interpretation of the urinary G:N ratio.

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# THE FOLIC ACID CONTENT OF BLOOD FROM VARIOUS SPECIES<sup>1</sup>

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The importance of one or more factors, collectively referred to as folic acid, in the nutrition of animals (including man) has become clearly evident during the last few years (1-11). Other reports (12-16) have included results on the occurrence of the active compounds in natural materials as well as the amounts in various tissues as influenced by the dietary intake (17-18). There is a paucity of information, however, on the amount of folic acid in the blood of different species. The amounts of folic acid in the blood before and after enzymatic digestion have been determined and are presented in this paper.

**EXPERIMENTAL.** Samples of blood were collected in oxalated tubes from humans, horses, cattle, pigs, chickens and turkeys. The samples were shaken thoroughly and an aliquot of whole blood was taken for folic acid analysis. Aliquots of whole blood or plasma were diluted with 5 volumes of water and assayed directly for their apparent free folic acid content. The method of Teply and Elvehjem (19) was used for the folic acid assays with *S. faecalis*<sup>2</sup> as the test organism. Synthetic folic acid<sup>3</sup> (*L. casei* factor) was used as the standard throughout this work. The apparent free folic acid in whole blood and plasma is shown in table 1. From the data it is apparent that in most species the level of folic acid is much greater in the whole blood than in the plasma. In fact the level of folic acid in the plasma of humans and cattle is so low that it could not be accurately measured. On the other hand the concentration of folic acid in the plasma and whole blood is approximately the same in the horse and pig.

Since a considerable difference was observed between various species in the folic acid content of whole blood and plasma, studies were carried out on some of the samples to determine if any of this vitamin occurred in bound form, unavailable to *S. faecalis*. One milliliter of whole blood or plasma was diluted with 15 ml. of distilled water. Ten milligrams of takadiastase were added in 1 ml. of 2.5 M sodium acetate and sufficient 0.1 N HCl added to adjust the pH to 4.5. The samples were then incubated for 16 to 18 hours at 37°C. Following incubation, the digests were autoclaved for 15 minutes to inactivate the enzyme, cooled, neutralized, diluted to 25 ml. and filtered. Aliquots were then taken for assay. To rule out the possibility that liberation of folic acid in the diluted

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<sup>2</sup> Formerly referred to as *S. lactis* R.

<sup>3</sup> Kindly supplied by Lederle Laboratories, Inc. This compound has the same potency as the isolated compounds (Bc and SLR factor) for *S. faecalis* (20) and is also active for animals.

samples occurred on standing, representative samples from each experiment were stored in the refrigerator for 16 to 18 hours and reassayed along with the samples digested enzymatically. Little change was noted in samples stored in this manner, while samples treated enzymatically showed a marked increase in folic

TABLE 1

*Apparent free folic acid in whole blood and plasma of various species (values expressed as mγ per ml.)*

SOURCE OF SAMPLES	NO. OF SAMPLES	WHOLE BLOOD		PLASMA	
		Range	Average	Range	Average
Human.....	7	0.5- 1.3	0.85	<0.5	
Chicken.....	9	4.4-16.5	8.7	1.4- 4.2	3.1
Turkey.....	12	10.2-27.6	16.8	2.5-10.8	6.0
Horse.....	6	2.2- 6.0	3.3	2.5- 4.8	3.5
Cattle.....	6	0.6- 4.5	1.9	<0.5	
Pig.....	5	4.5- 9.9	6.6	4.8- 8.7	6.1

TABLE 2

*Effect of enzymatic treatment on the folic acid content of whole blood and plasma (values expressed as mγ per ml.)*

SOURCE AND SAMPLE NO.	WHOLE BLOOD		PLASMA	
	Before enzymatic treatment	After enzymatic treatment	Before enzymatic treatment	After enzymatic treatment
Human 1	0.5	36	<0.5	39
2	1.1	38	<0.5	48
3	0.6	38	<0.5	34
4	1.1	41		
Chicken 1	7.5	22		
2	9.4	25	2.9	30
3	7.8	43		
4	4.4	38	1.4	42
5	6.9	25	2.7	35
Cattle 1	2.1	21	<0.5	22
2	4.5	29	<0.5	18
3	1.8	30	<0.5	20
4	0.8	21	<0.5	20
Pig 1	7.2	31	6.6	21
2	9.9	23	8.7	18
3	4.8	24		

acid activity as measured by *S. faecalis* (table 2). Correction for the folic acid content of the enzyme was made in all cases.

RESULTS AND DISCUSSION. Wide differences and considerable variation were noted in the apparent free folic acid content of whole blood and plasma from various species. Thus the amount in the whole blood or plasma of humans or cattle averaged less than 2 millimicrograms per ml. In contrast to these results

from 4 to 8 times as much was found in the whole blood from pigs, chickens and turkeys with the amount in horse blood intermediate between these groups. Plasma from the pig and turkey contained the greatest amount of free folic acid, an average of 6.1 and 6.0 m $\gamma$  per ml. respectively, while the amounts in the plasma of the horse and chicken averaged 3.5 and 3.1 m $\gamma$  per ml. Human and cattle plasma contained less than 0.5 m $\gamma$  per ml. It appears, at least in the case of the chicken and turkey, that more free folic acid occurs in the cells than in the plasma.

After samples of whole blood were digested with takadiastase, a marked increase in the folic acid content was noted (table 2). This was particularly true for human and cattle blood. The amounts of folic acid found after enzymatic digestion were quite similar for the four species studied (20-40 m $\gamma$  per ml.) with human blood slightly higher than cattle or pig blood. The values for plasma after enzymatic digestion were similar to those for whole blood after the same treatment. This represented from 2 to at least a 50 fold increase in the values for plasma and whole blood; the actual magnitude of the increase depended on the species considered. Apparently this vitamin is largely bound to plasma and cellular proteins and is unavailable to *S. faecalis* until treated enzymatically.

The use of techniques such as these should be valuable for identifying a folic acid deficiency in various animals, including man, if the amount in blood is influenced by the dietary intake. Furthermore the response in formation of red blood cells associated with folic acid therapy (8-11) may bring about a concordant rise in the folic acid content of the blood. Preliminary evidence indicates that the free folic acid content of the blood can be altered markedly by the amount in the diet of the turkey.

#### SUMMARY

1. The amount of folic acid in whole blood and plasma of various species has been determined before and after enzymatic digestion.
2. The amount of apparent free folic acid in whole blood for the human, chicken, turkey, horse, pig and cattle averaged 0.85, 8.7, 16.8, 3.3, 6.6 and 1.9 m $\gamma$  per ml. respectively. The amount in plasma also varied with different species.
3. A marked increase in the folic acid content of whole blood and plasma was observed after enzymatic treatment. From 20 to 40 m $\gamma$  per ml. of folic acid was found after this treatment.

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# THE INFLUENCE OF SOME ORGANS ON THE PYRUVATE LEVEL IN THE BLOOD<sup>1</sup>

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In 1928 it was shown independently in two different laboratories (1, 2) that the liver could remove lactic acid from the blood stream and pour glucose into it. Additional work revealed that two of the conditions necessary for this removal of lactic acid were a raised level of blood lactate and the postabsorptive state (3). Because of the metabolic relationship of lactate to pyruvate, it was decided to examine the effect of the liver, muscle and intestine on the pyruvate concentration in the blood. Since vitamin B<sub>1</sub> is necessary for the oxidation of pyruvate (4), thiamin deficiency might be expected to influence the pyruvic acid balance of various organs, and for that reason studies were made in normal and vitamin B<sub>1</sub> deficient animals.

**METHOD.** Two groups of dogs were used: one of 5 animals fed a complete diet and the other of 11 animals given a diet lacking only in vitamin B<sub>1</sub> (5). The members of the second group were studied in various stages of vitamin B<sub>1</sub> deficiency, some with gastrointestinal signs but before they had acute neurological changes, others when they displayed leg stiffness, leg weakness, and a few in the preconvulsant stages, or following convulsions. The normal animals were without food for 24 hours and the avitaminotic ones had been eating little for many days before sacrifice. The animals were always placed under pentobarbital anesthesia, 35 mgm./kgm. in the normal animals and less in the avitaminotic ones, in accordance with their impaired resistance to the drug. A median abdominal incision was made, the femoral triangle was exposed, and blood samples were drawn from the femoral artery and vein simultaneously and immediately thereafter from the hepatic and portal veins in the order given, 2 cc. samples were analyzed for pyruvate (6) and 1 cc. for lactate (7) and glucose (8). Glycogen determinations (9) were made of samples of liver at the termination of each experiment.

**RESULTS.** The results are presented in table 1, A and B. It may be seen that in the normal animal, the liver removes pyruvate from the blood stream just as consistently as it absorbs lactate, while glucose, on the contrary, is added to the blood stream. In the vitamin B<sub>1</sub> deficient animals, the liver still continues

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to extract pyruvate and lactate from the blood and to release its quota of glucose. The intestines pour out pyruvate and lactate and do so irrespective of the nutritional status of the animal. Muscle, whether in the control or avitaminotic animals, eliminated lactate but the result with pyruvate was less consistent. When however, the findings of the dogs exhibiting neurologic signs are considered separately from those on the other deficient animals, table 2, it may be seen that their muscles add pyruvate to the blood stream. The comparative effects of muscle, intestine and liver on blood pyruvates of normal and avitaminotic dogs exhibiting neurologic signs are shown in table 2. The figures in parentheses are the number of observations. In addition the first column presents the average of all the results in which pyruvate was added to the blood stream by the muscle, intestine and liver; the second contains the average values

TABLE 1

*The effect of liver, intestine, and muscle on the pyruvate, lactate, and glucose of the blood*

ORGAN	LIVER			INTESTINE			MUSCLE		
	Added (+)	No change (0)	Absorbed (-)	Added (+)	No change (0)	Absorbed (-)	Added (+)	No change (0)	Absorbed (-)
A. Dogs on adequate diet									
Pyruvate.....	4	4	19	12	12	3	9	7	7
Lactate.....	2	7	17	10	15	2	20	4	0
Glucose.....	16	1	2	5	6	7	3	3	9
B. Dogs on thiamin deficient diet									
Pyruvate.....	1	2	13	12	2	2	6	4	6
Lactate.....	2	1	11	11	3	0	10	1	1
Glucose.....	11	3	2	2	7	7	2	9	5

(+) Number of observations in which substance was added to blood, (0) in which there was no significant change, (-) in which substance was absorbed by liver, intestine or muscle.

for the absorption of pyruvate by these organs and the third shows the balance for all significant observations. It may be seen that the characteristic effect of the normal liver to absorb pyruvate is continued and intensified in vitamin B<sub>1</sub> deficiency as is the release of pyruvate by muscle and intestine. The average value for hepatic glycogen is 3.6 per cent in the control dogs and 1.6 per cent in thiamin deficient dogs.

DISCUSSION. It is known under certain conditions recovery from work in part may occur in the liver because of the glucose-lactic acid cycle between muscle and liver. From the present observations, we find that not only muscle and intestine lactate but also pyruvate are absorbed by the liver and presumably changed to carbohydrate. The liver of the dogs in vitamin B<sub>1</sub> deficiency absorbs pyruvate and lactate from the blood as it does in the normal. This observation is in accordance with the present viewpoint that thiamin is not ne-

cessary for the various steps of the Embden-Meyerhof scheme between glycogen and pyruvic acid but rather for the oxidation of pyruvic acid.

Since blood flow was not determined no deductions of a quantitative nature can be made in comparing the normal with the deficient animal. The observations of intestine and muscle however do not exclude an increased elimination of pyruvate. It has recently been shown that the normal brain adds small but significant amounts of pyruvate to the blood stream (10). This amount may be increased in vitamin B<sub>1</sub> deficiency. The only organ which

TABLE 2  
*Effect of organs on pyruvate of blood*

(a) Dogs on adequate diet			
			<i>Balance</i>
Muscle.....	<b>+0.39</b> (9)	<b>-0.42</b> (7)	<b>+0.04</b> (16)
Intestine.....	<b>+0.38</b> (12)	<b>-0.31</b> (3)	<b>+0.24</b> (15)
Liver.....	<b>+0.37</b> (4)	<b>-0.51</b> (.9)	<b>-0.36</b> (23)
(b) Dogs exhibiting neurologic signs of thiamin deficiency			
Muscle.....	<b>+0.62</b> (6)	<b>-0.40</b> (3)	<b>+0.29</b> (9)
Intestine.....	<b>+0.29</b> (11)	<b>0</b> (0)	<b>+0.29</b> (11)
Liver.....	<b>0</b> (0)	<b>-0.98</b> (13)	<b>-0.98</b> (13)

Figures in bold type indicate averages: those in parentheses, the number of observations.

Values following (+) are average for all observations in which organs added pyruvate to blood: following (-) for observations in which pyruvate was absorbed from blood, and under balance the average for all significant observations.

exhibits a qualitative change from absorption to elimination of pyruvate and lactate in a thiamin deficient animal is the heart (11).

The liver removes more pyruvate per 100 cc. of blood in the animals with neurologic signs than in the normal dogs. This indicates augmented hepatic function and may be correlated with the lower level of glycogen in avitaminotic animals. It is known that impaired hepatic glycogen favors the absorption of lactate and now it would seem that this conclusion may be extended to include pyruvate. Another factor for the greater hepatic utilization of pyruvate is the hyperpyruvemia existing in the deficient animals. Again this is similar to the augmented hepatic absorption of lactic acid with lactic acidemia. The low

level of glycogen, despite the accelerated absorption of pyruvate and lactate may be accounted for by a more rapid out-pouring of hepatic glucose.

#### SUMMARY AND CONCLUSIONS

Dogs fed an adequate diet and examined under pentobarbital anesthesia in the post-absorptive state reveal a pyruvate cycle resembling that of lactate between the liver on one side and muscle and intestine on the other, i. e., hepatic glucose is exchanged for muscular and intestinal pyruvate. In vitamin B<sub>1</sub> deficiency, for the most part, the patterns remain the same, the liver continuing to exchange glucose for the carbohydrate-split products of muscle and intestine. However, quantitative differences due to vitamin B<sub>1</sub> lack in the influence of these organs on blood pyruvate are not excluded by these studies but are suggested by the greater arteriovenous differences as well as the lower level of hepatic glycogen in the thiamin deficient animals.

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# THE EFFECTS OF WATER DIURESIS AND EXERCISE ON THE VOLUME AND COMPOSITION OF THE URINE

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During a class experiment made in October 1943, it was found that results obtained differed from those reported by Eggleton (9). We therefore decided to reinvestigate the effects of water intake on urinary volume, pH and chloride excretion, and in addition, to make further investigations of the effects of exercise on urinary secretion. The experiments may be conveniently divided into groups: 1. The effects of increased water intake on urinary volume, pH and chloride excretion, and the influence of meals and time of day on these results. 2. The effects of exercise on the course of a water diuresis and the influence of time of day, etc., on the changes produced. 3. Renal dynamics during diuresis and exercise, (a) glomerular filtration rate, (b) renal blood flow.

1. EFFECTS OF DIURESIS. *Methods.* These experiments were performed on 42 subjects who emptied the bladder and collected two samples of urine at 15 minute intervals to obtain an estimate of the basal urine flow. Eight hundred cubic centimeters of water was then drunk and urine samples collected every 15 minutes until the rate of urine flow had returned to basal levels. Nineteen experiments were performed in the morning, 10 on subjects who ate no breakfast and 9 on subjects who ate a normal breakfast. The taking of a meal did not appear to influence the results obtained and so the 23 subjects who did afternoon experiments had their usual lunch before arriving at the laboratory at 2 p.m. Samples were analysed for chloride by a modified Volhard technique (19) and for phosphate by Briggs' method (18). pH was determined by the glass electrode on the Cambridge pH meter with frequent standardisation against buffer. Precautions were taken to prevent loss of CO<sub>2</sub>. Haematocrit values were determined by the method of Meyerstein (14).

*Results. Changes occurring during diuresis. (a) Urine output.* The average basal urine flow in the pre-diuretic samples was 1.03 cc./min. (range 0.17–2.06 cc./min) in the morning, and 0.77 cc./min. (range 0.33–1.35 cc./min) in the afternoon. The amount of urine passed during diuresis showed great variation, ranging from 191–1174 cc. In one subject, 1300 cc. of water failed to provoke a diuresis. The average volume of fluid recovered during the actual diuresis was 711 cc. in the morning experiments (18 trials) and in the afternoon experiments 700 cc. (23 trials). Diuresis was taken as an increase of at least 42 per cent above basal level in rate of urine flow (2). An attempt was made to assess the degree of hydration of the subjects on the basis of Adolph's statement that duration of diuresis is related to water load, and that the volume recovered during a fixed time after drinking water is greater in well hydrated individuals. In the morning the diuresis lasted 1 hour 45 minutes (19 trials) and 1 hour 42 minutes in the

afternoon series (23 trials). The amount of water excreted during the first  $1\frac{1}{2}$  hours after drinking the 800 cc. of water averaged 552 cc. in the morning experiments (30 trials) and 526 cc. in the afternoon (21 trials). This period, of course, included the latent period before diuresis began. Results obtained showed no sex difference.

(b) *pH*. All urines of pH 6.9 upwards showed a fall, and more acid urines a rise of pH as the urinary volume increased (7). Two typical examples are shown in figure 1. In 11 control experiments, in which no water was taken, pH fell in subjects with urines of high pH, and rose in those with more acid urines during the course of the morning. In some subjects, urinary pH was higher when breakfast was taken than after fasting; in others, the reverse was the case.

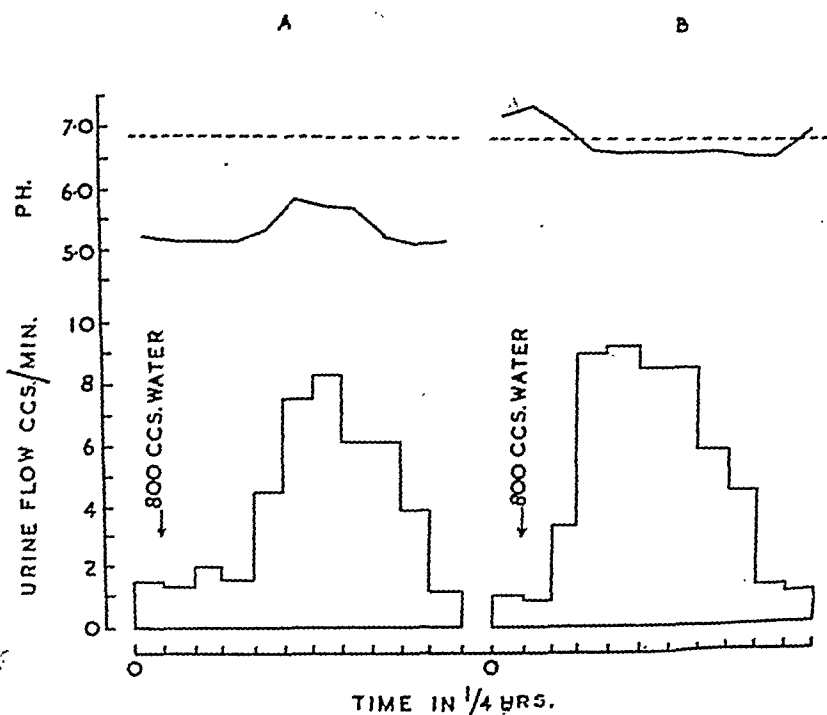


Fig. 1. Changes in urinary pH during a simple water diuresis in two subjects

(c) *Chloride excretion*. In control experiments, the chloride concentration of the urine remained remarkably constant, and graphs of total chloride excretion mirrored the volume curves. In 17 of 36 subjects, there was a rise in chloride excretion at the beginning of diuresis, followed by a fall to the initial level, or below the initial level at maximum diuresis. In some subjects (fig. 3), this initial rise preceded the diuresis (9). In a few subjects, chloride excretion remained below the initial level after the diuresis had passed off (fig. 2A and C). In 12 subjects, chloride excretion fell during the diuresis (fig. 2A and B); in 2, there was a prolonged slight rise during diuresis returning to the initial level as this wore off (fig. 2D). In 5 subjects, the chloride excretion rose with the increased urinary volume (fig. 2C).

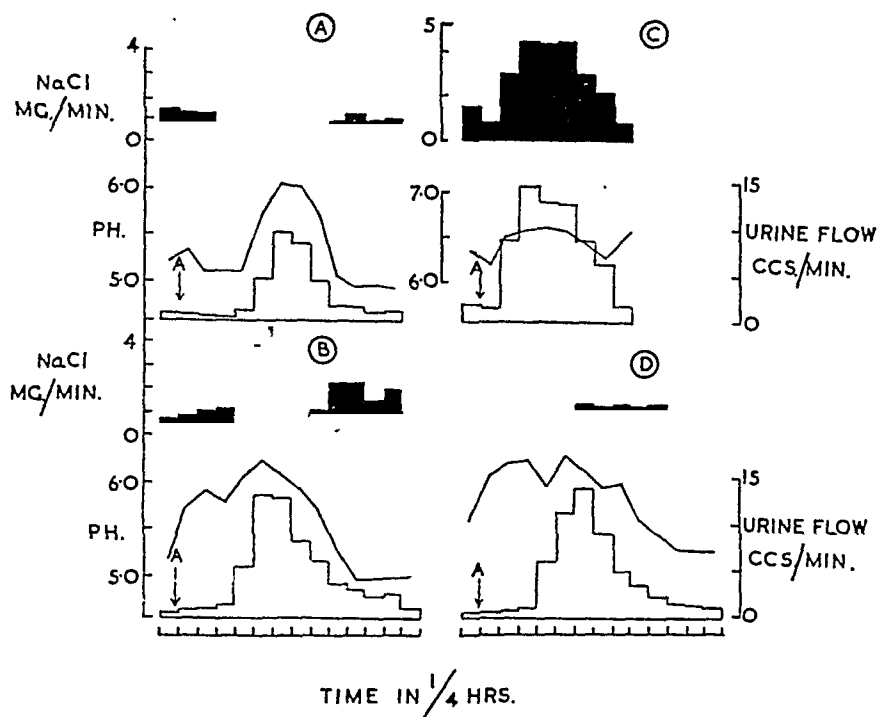


Fig. 2. Changes in urinary pH and chloride excretion during a simple water diuresis in four subjects. Eight hundred cubic centimeters water taken by mouth at A.

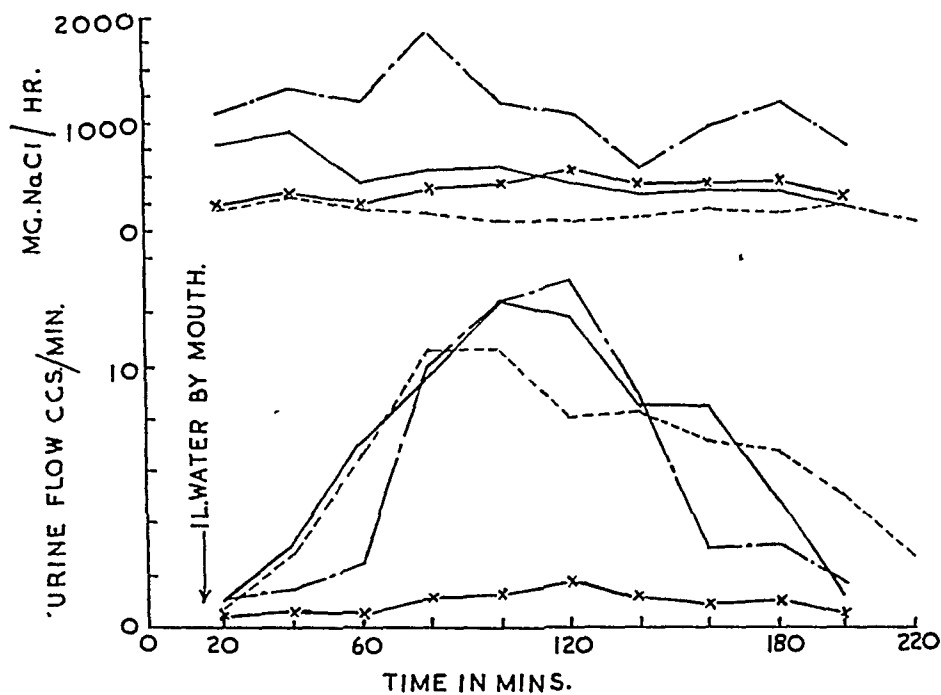


Fig. 3. The effects of previous high and low salt intakes and low water intake on chloride and water excretion in one subject. X—X, low water intake. - - - - -, high salt diet. . . . ., low salt diet. —, control.



(d) *Variations in chloride and fluid intake.* The subject, a male medical student aged 18 years, on five successive days, drank 800 cc. of water and collected urine as described above. On each occasion chlorides fell during diuresis after a slight rise at the onset, the curves being perfect replicas (fig. 3—Control). After a low chloride diet similar results were obtained, the chloride excretion being at a lower level. After high salt intake chloride excretion was at a higher level although the shape of the curve was similar to the control. After previous low fluid intake both chloride and water output were on a reduced level, the chloride excretion following that of water.

2. EFFECTS OF EXERCISE. *Methods.* Fifty trials of the effects of exercise were performed, 30 in the morning and 20 in the afternoon. Diuresis was produced, as in Section I, by drinking 800 cc. of water and exercise, consisting of running  $\frac{1}{4}$  mile over rough ground at full speed, was taken as soon as diuresis was established.

*Results.* (a) *Urine output.* In the morning, an inhibition occurred in all but 1 of the 30 trials. Seventeen trials were performed by subjects who took no food, but drank a cup of tea on rising, thirteen trials after a normal meal, and since it appeared that taking a meal had no significant effect on the results obtained, the subsequent afternoon experiments were performed after a normal lunch. In these experiments (20 subjects) however, only 9 subjects showed inhibition of diuresis and in only 2 of these was the effect at all marked. In an earlier series, 25 experiments had been performed in the afternoons with more severe exercise, running  $\frac{3}{4}$  mile or exercising to exhaustion on the bicycle ergometer. There was no inhibition in 4 of these subjects, slight inhibition in 9, moderate inhibition in 7, and marked inhibition in 5. In subjects in whom inhibition of diuresis did occur, the maximum fall in urine volume was frequently not attained until  $\frac{3}{4}$  to 1 hour after cessation of exercise. Thus, severe exercise in the afternoon was not so effective in producing inhibition as more moderate exercise in the morning. Notes were made of the size and composition of meals and the differences in response of morning and afternoon subjects could not be related to different types of meal.

The average time taken to run  $\frac{1}{4}$  mile did not differ significantly in morning and afternoon subjects. There was no evidence that afternoon subjects were not making their maximum effort.

The proportion of water drunk, which was recovered by diuresis, was less in subjects taking exercise than in the same subjects when water was drunk and no exercise taken. The average volume of urine passed during diuresis plus exercise in the morning was 597 cc. (30 subjects) compared with 711 cc. in controls (19 subjects). In the afternoon, 569 cc. of urine was the average volume passed by 20 subjects compared with 700 cc. in controls (23 subjects).

The duration of diuresis in the morning experiments was 2 hours 40 minutes (28 trials) and, in the afternoon series, 1 hour 55 minutes (20 trials).

(b) *pH.* In every trial, the pH of the urine fell sharply as a result of taking exercise (fig. 4). Before exercise, the pH range was 7.36 to 5.70, with a mean of 6.3; after exercise, the pH fell to a range 4.49 to 5.46 with a mean of 4.94,

the greatest fall being from 7.36 to 4.85. In those subjects showing an inhibition of urinary volume, the pH change often preceded the volume change and began to rise again half-an-hour after the exercise, even though the urinary volume was still falling. The rise continued as the diuresis reappeared, and there was a fall as this wore off (fig. 4D). The taking of a meal did not influence the rate at which pH began to rise after exercise.

(c) *Chloride excretion.* In every case, no matter what the pattern of chloride excretion during a simple water diuresis, the total urinary chloride output fell as a result of exercise. This change too, was frequently apparent before the volume change (fig. 4D). After about an hour, chloride excretion rose again

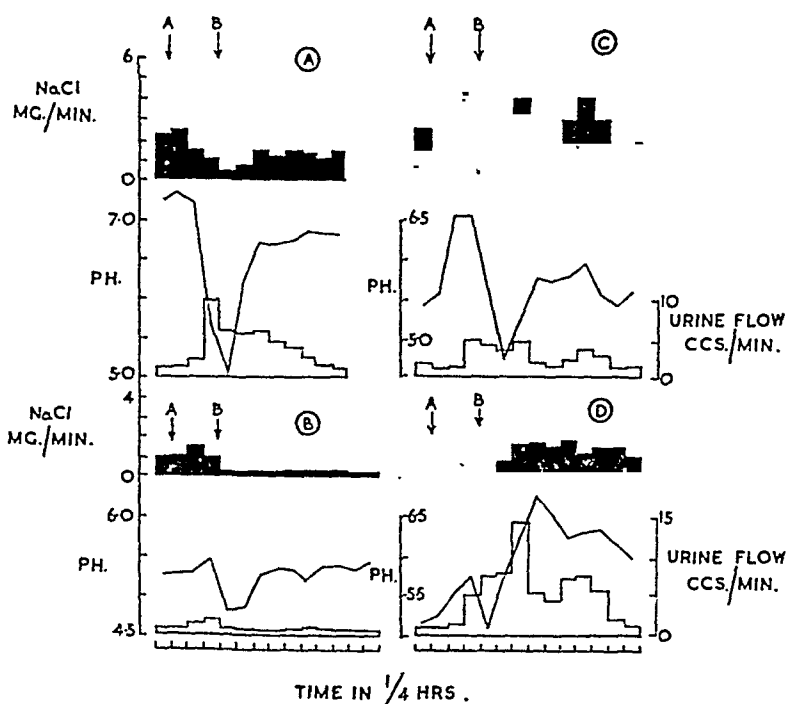


Fig. 4. Effect of diuresis and exercise on urinary pH and chloride excretion in four subjects. Eight hundred cubic centimeters water by mouth at A. Exercise taken at B.

only in those subjects who showed increased chloride excretion during water diuresis.

3. RENAL DYNAMICS DURING DIURESIS AND EXERCISE. *Methods.* In order to obtain a clearer picture of the changes occurring during diuresis, and its subsequent inhibition by exercise, renal function was studied in more detail on 10 students (9 male and 1 female).

The subjects took 800 cc. of water by mouth, or more if necessary to produce a diuresis, and as soon as diuretic urine samples were obtained, urine collection in 15 or 20 minute periods was begun. Blood samples were taken during the experiment and clearances determined in the usual way. Filtration rates were determined either by inulin or mannitol clearance, and renal plasma flow by the diodone method (10). When diuresis was at its height, the subject ran  $\frac{1}{4}$  mile

at full speed. Urine samples continued to be collected until the diuresis returned after the inhibition and where there was no inhibition, until the end of the diuresis.

(a) *Glomerular filtration rate.* During the diuresis, the filtration rate ranged from 70 to 156 cc./min., the average being 112 cc./min. When exercise was taken, there was a marked fall in filtration rate, values in these periods ranging from 48 to 91 cc./min. with an average of 62.2 cc./min., a fall of 44.6 per cent (fig. 5).

(b) *Renal plasma flow.* In the pre-exercise periods, the renal plasma flow averaged 709 cc./min. (range 483-976 cc./min.). During exercise, the average plasma flow averaged 495 cc./min. (range 249-670 cc./min.) a fall of 30 per cent. Renal plasma flow in one subject fell 34 per cent during exercise with no change

TABLE 1

SUBJECT	GLOMERULAR FILTRATION RATE (CC./MIN.)			RENAL PLASMA FLOW (CC./MIN.)		
	Before exercise	During exercise	After exercise	Before exercise	During exercise	After exercise
F. L. R.	150	48	110	615	185	515
J. C.	124	84	151	555	368	402
M. A. C. J.	70	48	103	1,150	622	885
D. F. C.	144	50.9	129	538	249	602
E. S. R.	156	78.4	85.7	609	365	380
J. M. A. C. S.	103	41.4	73.7	670	400	590
J. R. C.	123	91	120	864	663	514
H. J. E. C.	86.5	55	73.5	685	500	670
A. G. J.	83	58.5	51	940	670	586
Range.....	70-156	44.4-91	51-151	538-1150	185-670	40-885
Mean.....	115	62	99	736	447	579

in urine flow, whilst in another, urine flow doubled although there was a 40 per cent fall in plasma flow.

*Filtration fraction*  $\left( \frac{\text{filtration rate}}{\text{renal plasma flow}} \right)$  in the pre-exercise diuresis averaged 15.8 per cent whilst in exercise it fell to 12.6 per cent, a fall of 20 per cent.

**DISCUSSION.** During class experiments on effects of exercise on diuresis, it was noted that inhibition of urine flow did not always occur, as opposed to the findings of Eggleton (9) Wilson, Long, Thompson and Thurlow (21). A re-investigation of the whole subject of water diuresis and that of the effect of exercise upon it, was therefore undertaken.

It was thought possible that the state of hydration might account for the failure of inhibition of urine flow in some subjects. According to Adolph (1, 2), the degree of hydration of a subject may be assessed by the percentage of water drunk, which is recovered by diuresis, and also by the rate at which this water is excreted. In our series, the volume of urine passed during the first 1½ hours

after drinking 800 cc. of water, averaged 552 cc. in the morning and 526 cc. in the afternoon. On Adolph's criteria, therefore, there is no evidence for the suggestion that difference between morning and afternoon experiments could be due to variations in hydration (7). It was also noted that the basal urine flow was no guide to the extent of diuresis, or whether in exercise an inhibition would occur.

As has been stated above, some students failed to exhibit a diuresis following 800 cc. of water, although if fluid was pushed, a diuresis eventually occurred. The frequency of such a finding is usually 2 to 3 students out of 80 every year. It is pertinent to note that some of this small group of students state that they always feel thirsty. Although these individuals would appear to be somewhat

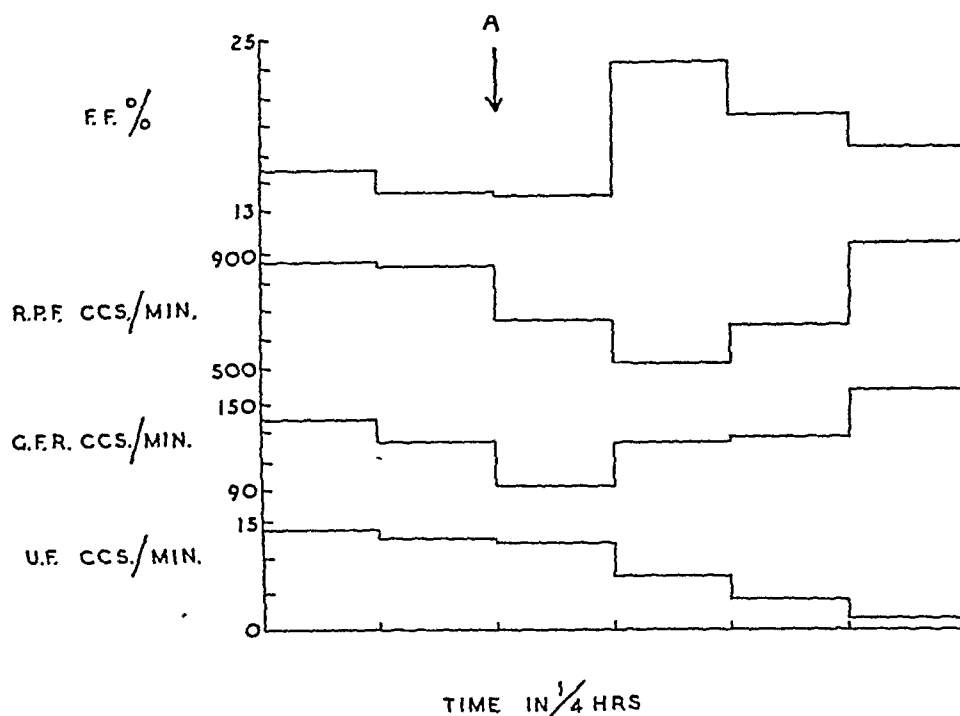


Fig. 5. Renal dynamics during exercise for one typical subject (J. R. C.). Exercise taken at A, diuresis established by eight hundred cubic centimeters water by mouth.

dehydrated, until some more absolute criteria of minor degrees of fluid deficiency are found, the suggestion must remain hypothetical. Once these individuals receive sufficient water to establish a diuresis, their behaviour to subsequent exercise does not appear to differ from their fellow-students, and when examined by renal function tests, showed no abnormality of renal dynamics.

During diuresis, the pH showed much variation; the pH of the urine did not move towards that of the plasma but tended to move upwards or downwards to pH 6.9. This might be correlated with the fixed pH found in chronic nephritis, usually around 6.8.

Even at maximum diuresis, the urine was still well differentiated from plasma. There was no evidence of the constant occurrence of an alkaline tide in our subjects, a similar finding to that of Barnett and Bloome (8).

In a number of experiments, both pH and chlorides started to move often  $\frac{1}{4}$  hour after taking water. Examination of the figures set out by Wilson et al. (21) showed the same phenomenon. The experiments in which the water and chloride intake varied, appeared to support the suggestion that chloride excretion during water diuresis is dependent on the dietary habits of the subjects. The most common response was that as reported by Eggleton (9), of a fall in chloride excretion during diuresis which may be preceded by the preliminary rise. Eggleton (9) reported a decreased chloride excretion during water diuresis confirming the results of earlier workers, (15, 3, 13), and classed as "abnormal" one individual who showed an increased chloride excretion during diuresis. It would appear from our results that increased output of chloride during water diuresis is a fairly common occurrence. McCance and Young (12) in experiments on the rehydration of dehydrated persons, showed that excretion of water and chloride in the urine is closely inter-related. It may be that the varying effects of water intake on the excretion of chlorides in urine depend on the different concentrations of chloride in the body fluids, possibly due to the dietary habits of the individuals concerned.

In exercise, in all subjects, no matter what the pattern of chloride excretion during a simple water diuresis, the total urinary chloride output fell sharply as a result of exercise. This change, too, was frequently apparent before the volume change, where such a change occurred. The fact that chloride excretion may fall before volume decreases, is also evident from the results of Wilson et al. (21).

The changes in blood flow during exercise are of interest. In all the subjects examined, the filtration rate decreased but did not diminish as fast as the blood flow; possibly the kidney prefers to maintain filtration even at the price of diminishing blood flow. This diminution of blood flow might be profound and yet have no significant effect on the course of the diuresis.

In the light of Lampport's (11) interpretations, it would appear that we are dealing with changes in both afferent and efferent vessels to the glomerulus. We postulate that the primary change is a constriction of the afferent arterioles and that following on this constriction, the change in the efferent arterioles occurs in order to maintain filtration in the face of the falling renal blood flow. This would suggest that the body is shunting blood from the kidneys to the active muscle, the amount involved being over 500 cc. blood/min. in some cases. Since the findings on the circulation of the kidney in exercise show some similarity to those of the kidney in hypertension, an exact assessment will be of importance.

We have examined chloride excretion and reabsorption in those subjects for whom we had filtration data and obtained values for their renal tubular activity as described by Barclay and Kenney (5, 6). In figure 6 is illustrated such an analysis for chloride excretion and reabsorption in 3 typical cases. We have plotted the absolute amount of electrolyte reabsorbed, the clearance, the threshold (4) and the index E, together with pertinent renal dynamic data.

In figure 6 is illustrated the analysis of chloride reabsorption in 3 typical cases. Plasma level may either rise (figs. 6A and 6B) or fall (fig. 6C). Chloride clearance may either rise or fall with diuresis, as already mentioned, and appears to be in no way correlated with filtration rate. The amount of chloride reabsorbed, however, mirrors to some extent filtration rate. The kidney thus appears to be able to deal with an increase in the amount filtered in a quantitative fashion. As a result, threshold mirrors in a fairly precise manner plasma level. The threshold may determine the amount reabsorbed whilst activity remains constant or activity may be the determining factor. These facts could most easily be explained by postulating two components to the reab-

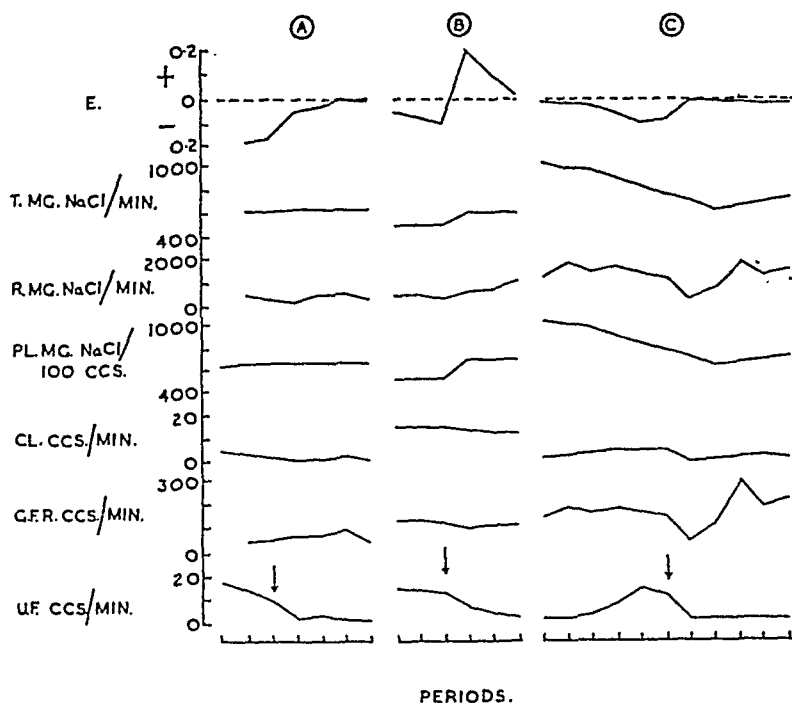


Fig. 6. Effect of diuresis and exercise upon chloride excretion and reabsorption in three subjects. Diuresis established by eight hundred cubic centimeters water by mouth. Exercise taken at A.

sorptive mechanism, proximal and distal, as has already been done by Rehberg (16) Shannon (17) Walker, Bott, Olive and MacDowell (20). It would appear reasonable to suppose that threshold is mainly concerned with the proximal reabsorption which may be purely iso-osmotic and that the activity reflects changes occurring in the distal component, since a quantitative change in the iso-osmotic reabsorption would appear as a threshold change and give no change in activity.

In some subjects, as was noted by Eggleton (9), there is a preliminary rise of chloride excretion and an attempt may be made to analyse the factors involved. The subject already described (fig. 3) consistently showed such an increased chloride excretion before the diuretic effect on water intake was seen. As a re-

sult of taking water, threshold falls and no change takes place in activity, i.e., an increased fraction of reabsorption is occurring iso-osmotically, presumably in the proximal tubule, and chloride excretion rises. Almost at once, a change occurs in activity, the distal mechanism counteracting the fall in threshold and the chloride excretion once more falls. In this series of experiments, the first change to occur is in threshold, activity changing after a period of up to 30 minutes (fig. 6). This change in activity tends to counteract the threshold change but almost always fails to do so quantitatively and the amount of chloride excreted rises. Some subjects, however, appear to be able to increase tubular activity enough to overcome the threshold change and, indeed, increase so much as to reduce chloride excretion.

When diuresis is inhibited by exercise, activity falls off at once and this change is also reflected in threshold. One difficulty is that threshold is the sum of both proximal and distal components and only when the distal tubule is inactive will changes in threshold indicate happenings in the proximal tubules alone. It thus appears that there exists in the kidney some mechanism for bringing into action the active reabsorption component of the distal tubule at the onset of diuresis and this is cut off as soon as the urine flow is inhibited by exercise.

Chloride normally appears to be dealt with by an active, presumably distal mechanism, and the results suggest that on the inhibition of diuresis by exercise, there is a fall in activity towards chloride, the kidney reverting to the slightly active method of reabsorption which held before diuresis was set up.

#### SUMMARY

1. Results are presented of experiments carried out over three years on the changes occurring during diuresis and the effects of exercise, time of day, and meals on these changes.

2. As a result of exercise, water diuresis may or may not be inhibited.

3. pH of the urine during diuresis tends towards 6.9 irrespective of initial level; during exercise there was always a marked fall.

4. It has been found that there is always a fall in plasma flow, in filtration rate and a rise in filtration fraction during exercise.

5. As a consequence of taking water, there is either a rise or fall in chloride excretion. It is shown that this is a reflection of the varying degrees of tubular activity towards chloride, and as a result of exercise, tubular activity increases still further and there is a marked fall of chloride excretion.

6. Our findings offer support for the postulate that there is a proximal and distal component to reabsorption.

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# THE RÔLE OF THE VAGUS NERVES IN THE INHIBITION OF GASTRIC MOTILITY BY FAT AND BY INTESTINAL AND URINARY EXTRACTS

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Extracts of upper intestinal mucosa have been repeatedly demonstrated to be able to inhibit the hunger motility and the motility induced by distention in the intact stomach. It has been presumed that the agent responsible for this inhibition may be related to the chalone enterogastrone<sup>2</sup> which is released by the upper intestinal mucosa when fat is in contact with it.

A further step necessary for the establishment of the relationship between motor inhibitory substances in intestinal extracts and the chalone released by the presence of fat in the intestine is the demonstration that these extracts are able to act in the extrinsically denervated stomach. The studies here reported were undertaken in order to determine whether intestinal extracts, "enterogastrone" concentrates, and the analogous gastric inhibitory substance in urine, urogastrone, are able to inhibit the motility of the vagally denervated stomach.

The presence of fat in the upper intestine depresses both the secretory and the motor activity of the stomach. Similarly, extracts of intestinal mucosa and extracts of urine depress both motor and secretory activity *in the intact stomach*. Therefore, the question arises whether the secretory and motor inhibitions are caused by a single constituent of these extracts and furthermore whether the depression of these two types of gastric activity is due to the same or different chalones when fat is acting in the intestine. Evidence bearing upon these questions will be presented in this report.

The possible relationship between enterogastrone and urogastrone has been discussed by Gray (8).

**METHODS.** The assays of motility depression were performed on trained, unanesthetized dogs as described by Gray, Bradley and Ivy (2). A balloon in the stomach was attached to a water manometer by a rubber tube. The ma-

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<sup>2</sup> The term enterogastrone was originally coined by Kosaka and Lim (1) to designate the active ingredient(s) of intestinal extracts which depress gastric motility and secretion in a manner similar to the action of fat in the intestine. This definition presumes and the etymology of the term implies that the depressant ingredient or ingredients in these extracts are the same as or are related to the chalone released by the presence of fat in the intestine, a view not entirely unwarranted by the facts at hand. However, until more conclusive evidence on this point is available we prefer to restrict the use of the term enterogastrone to the chalone released by the intestinal mucosa when fat is in contact with it and to refer to intestinal extracts mimicking this action as enterogastrone-like concentrates or by the word "enterogastrone" in quotation marks to indicate the inconclusive status of the problem of the relation between the extracts and the naturally produced endogenous chalone. It will be recognized that this problem in terminology is general in nature and occurs in the consideration of many humoral and hormonal mechanisms.

nometer writing arm recorded gastric motility and tonus on a slowly revolving kymograph drum. Motility was initiated and maintained by distention of the recording balloon with air introduced through a T-tube. This distention type of motility was employed in all the studies here reported; hunger motility was not studied.

Each inhibiting agent was tested in both vagally innervated and vagally denervated stomach preparations. Normal intact dogs, trained to swallow the stomach tube with the recording balloon on its end and to lie still during the experiment, were used for the studies on the vagally innervated stomach. Two types of vagotomized stomach preparations were employed. In the first type (3 dogs), the vagi were transected subdiaphragmatically and gastric fistulas established by installing metal cannulas in the stomachs. The second type of vagotomized stomach preparation was represented by 2 dogs with pouches of the entire stomach and esophago-duodenostomies. In the vagotomized preparations the balloons were introduced into the stomachs via the fistulas.

Fat, "enterogastrone" concentrates, and urogastrone preparations were assayed for their ability to depress motor activity in the vagotomized and the intact stomach. In each experiment only one inhibitory agent was administered, after a control period of approximately 30 minutes during which the distention induced motility was recorded. The fat test meal was prepared by adding sufficient cream to the yolk of one egg to bring the volume to 400 cc. Twenty-five or 50 cc. portions of this mixture were introduced into the stomach by a small stomach tube attached to and introduced with the tube leading to the recording balloon. In the total pouch dogs with esophago-duodenostomies the mixture was introduced directly into the duodenum by stomach tube.

Each "enterogastrone" and urogastrone preparation used in these studies had been previously assayed for its ability to inhibit histamine-induced gastric secretion and had been shown to be of standard potency in this regard. "Enterogastrone" and urogastrone preparations were administered intravenously.

On a number of occasions, proof of the mechanical integrity of the apparatus was demonstrated by the intravenous administration of small quantities of pituitrin which caused an immediate, transient loss of tone and depression of motility.

**RESULTS.** The average response in terms of tonus loss or gain and duration of the effect has been tabulated. It was considered justifiable to present the data in this manner because the response to a given dose of any one of the inhibitory agents was reasonably consistent and for the purposes of the present study we were interested in determining whether or not an agent inhibited motility and not in the magnitude of this effect. The results obtained on the normal dogs with the intact vagi are presented in table 1. The results obtained on vagotomized dogs are presented in table 2. For the sake of brevity, the data on motility per se have been omitted from the tables because depression of tonus was invariably accompanied by depression of contractions both as regards frequency and amplitude, and marked loss of tone was always accompanied by complete cessation of contractions.

*A. Egg-yolk and cream.* The introduction of 50 cc. of this mixture into the

vagally innervated stomach stopped motility and reduced tonus an average of 43 mm. of water for over two hours. Contractions ceased promptly and tonus decreased gradually. In both types of vagally denervated preparations the same quantity of egg yolk and cream caused cessation of motility and an average decrease in tonus of 33 mm. of water lasting for about one hour. It is thus

TABLE 1  
*Assays on vagally-innervated stomachs*

DRUG	DOSE	NO. OF ASSAYS	AV. TONUS DECREASE (MM. H <sub>2</sub> O)	AV. DURATION  min.
Egg yolk-cream.....	50 cc.	2	43	120+
Pituitrin.....	0.05 u	4	22	1.6
Pituitrin.....	0.1 u	5	36	3.9
Pituitrin.....	0.5 u	5	42	11.3
Urogastrone 62A4B.....	1 mgm.	6	33.3	5.3
Enterogastrone EG59.....	5 mgm.	17	46	5.2

TABLE 2  
*Assays on vagotomized stomachs*

DRUG	DOSE	NO. OF ASSAYS	AV. TONUS INCREASE (MM. H <sub>2</sub> O)	AV. TONUS DECREASE (MM. H <sub>2</sub> O)	AV. DURATION  min.
Egg yolk-cream.....	25 cc.	2		21	39
Egg yolk-cream.....	50 cc.	4		33	60
Pituitrin.....	0.05 u	4		37	9.6
Pituitrin.....	0.1 u	3		44	38.0
Pituitrin.....	0.5 u	1		48	23.0
Urogastrone 62A4B.....	2 mgm.	1	0	0	0
Urogastrone 62A4B.....	4 mgm.	1	0	0	0
Urogastrone 62A4B.....	6 mgm.	1	0	0	0
Urogastrone 62A5B.....	2 mgm.	1	76		21
Enterogastrone EG59.....	5 mgm.	4	9		1.5
Enterogastrone EG59.....	10 mgm.	9	81		3.5
Enterogastrone EG59.....	20 mgm.	5	45		5.0

evident that fat effectively inhibits gastric motor activity in both the vagally innervated and vagally denervated stomach.

B. "Enterogastrone" concentrates. An "enterogastrone" concentrate E G 59, prepared by the tannic acid procedure (2), resulted in an average tonus decrease of 46 mm. of water for an average duration of 5.2 minutes in 17 assays in normal dogs with intact vagi using a dose of 5 mgm. intravenously. In the vagally denervated animals, administration of the same extract in the same dose produced an average increase in tonus of 9 mm. of water for an average duration of 1.5

minutes in four trials. The stimulatory effect on the gastric tonus of the vagotomized animals was greater in amplitude and duration when 10 and 20 mgm. quantities were tested.

A further demonstration of the difference in the effect of "enterogastrone" concentrates on vagally innervated and vagally denervated stomach preparations was achieved in a single animal which had been provided surgically with both an innervated and a vagally denervated pouch. The description of the surgical procedure used in preparing this animal has appeared elsewhere (3). Injection of 50 mgm. of a tannic acid preparation of "enterogastrone" concentrate while recording motility simultaneously from the two pouches, resulted in augmented tone in the denervated pouch and depression of tone and motility in the innervated pouch.

These findings clearly demonstrate that the gastric motor depressant in the intestinal extracts used in these experiments is effective only in the vagally innervated stomach preparation.

A more highly refined "enterogastrone" concentrate, P<sub>1</sub>, prepared by the picric acid purification procedure (4), caused no alteration in motility in 5 trials on 2 dogs with intact vagi when exhibited in 20 mgm. doses intravenously. Inasmuch as this extract, like the tannic acid products, effectively inhibited histamine-induced gastric secretion, it is apparent that the gastric motor depressant in these intestinal extracts is either removed or destroyed by the additional chemical treatment of the picric acid procedure without affecting the gastric secretory depressing moiety.

C. *Urogastrone*. Preparation 62A4B, which effectively inhibited histamine-induced gastric secretion in 0.5 mgm. doses, produced an average tonus decrease of 33.3 mm. of water for an average duration of 5.3 minutes in 6 motor assays in dogs with normal vagally innervated stomachs using a 1 mgm. dose intravenously. Two, four and six milligrams of the same preparation were completely without effect on the motility of vagotomized stomach preparations. Another urogastrone preparation, 62A5B, prepared by the same method, caused a marked increase in tonus when injected intravenously in 2 mgm. doses in the vagally denervated animals.

These findings indicate that the gastric motor depressant in urine extracts, like that in intestinal extracts, is effective only in the stomach in which vagal innervation has been preserved.

D. *Pituitrin*. In both vagally innervated and vagally denervated stomach preparations pituitrin produced an abrupt cessation of gastric motor activity followed by rapid recovery. The drug was administered intravenously in doses ranging from 0.05 to 0.5 unit.

DISCUSSION. The significant findings in this study are: 1. The motor inhibitory factors in urogastrone and "enterogastrone" extracts are active only in the vagally innervated stomach. In the vagally denervated stomach these extracts are either without effect upon or are stimulatory to motor activity. It is to be recalled that these extracts exert their characteristic gastric secretory depressing action in both the vagally innervated and vagally denervated stomach. 2.

The motor inhibitory factor in intestinal extracts which is effective in the vagally innervated stomach can be separated chemically from the secretory inhibitory factor. It has merely been demonstrated that the gastric secretory depressant can be freed of the motor depressant; recovery of the motor depressant from the by-products of purification has not been attempted.

In previous studies on the inhibition of gastric motility by fat and by intestinal and urinary extracts some investigators have made their observations upon hunger motility whereas others have employed distention-induced motility. The possible difference in the genesis of these two types of motility has not been taken into consideration in the interpretation of data. It is important for the understanding of the discussion which follows that the distinction between hunger motility and distention-induced motility be borne in mind.

The belief that the inhibition of gastric motility by the presence of fat in the intestine is mediated by a humoral mechanism is based upon the observation of Farrell and Ivy (5), later confirmed by Quigley, Zettleman and Ivy (6), that the instillation of olive oil into the intestine inhibits the *hunger* contractions of the extrinsically denervated, subcutaneously autotransplanted gastric pouch. However, a similar inhibition of hunger motility in the isolated pouch can be produced by foods other than fat although the latent period for the action of fat is shorter than that for other foodstuffs. Therefore, although the inhibition of hunger motility by fat has been shown to be humorally transmitted, it has not been shown to involve a mechanism peculiar to fat. The nature of this humoral inhibition of hunger motility by food has not been determined. It is not, in the case of fat, due to the absorption of the products of digestion (6). Theoretically, it may be due to release of an inhibitory substance or to inhibition of the release of a hypothetical "hunger hormone".

The unique property of fat in regard to its effect on gastric motility is its ability to inhibit the *digestive* motility and thus delay gastric emptying, as originally noted by Ewald and Boas (7). Experimentally the motility induced by distention of the stomach probably corresponds to the normally occurring digestive type of motility. Inhibition of distention-induced motility in the vagally denervated stomach has not been studied hitherto. The reason for the failure to study this problem is that it is difficult to induce motility by distention in the vagally denervated stomach. We have found that motility can be induced regularly in the vagally denervated stomach if the distending balloon is placed in the pyloric portion; this precaution was taken in the experiments detailed above.

The evidence presented in this report constitutes the first demonstration of the ability of fat acting in the upper intestine to inhibit distention-type motility in the vagally denervated stomach. It remains to be demonstrated that this effect can be produced in the completely extrinsically denervated stomach pouch. The crucial experiment for the demonstration of the hormonal nature of the inhibition of the digestive type of motility by fat would consist of the inhibition of distention-induced motility in the transplanted gastric pouch. Transplanted pouches hitherto used have been fashioned from the fundic portion of the stomach and do not show a motor response to distention. It is probable

that a transplanted pouch of the pyloric portion of the stomach would show a motor response to distention, and thus would be a satisfactory preparation for the crucial experiment.

The demonstration that intestinal and urinary extracts fail to inhibit distention-induced motility in the vagally denervated stomach indicates that the motor inhibitory factor in these extracts which acts in the vagally innervated stomach is not related to the motor inhibitory chalone released by the presence of fat in the intestine. It may be nonspecific in character. There is a distinct possibility that there exists a specific motor inhibitory substance in intestinal mucosa capable of inhibiting distention-induced motility in the extrinsically denervated stomach and that it has never been extracted. Efforts should be directed toward demonstrating such a substance.

The finding that the motor inhibitory effect of intestinal extracts, unlike the secretory inhibitory activity, can be produced only in the vagally innervated stomach plus the finding that intestinal extracts can be freed of this motor inhibitory factor by chemical purification indicates that the motor and secretory inhibitory factors in these extracts are separate substances. This leads to the conclusion that either (a) separate chalones are elaborated by the intestinal mucosa when fat is in contact with it, one depressing secretion and the other motility (assuming that only the former has thus far been extracted), or (b) a single chalone inhibiting both secretion and motility is released by the intestine when fat is in contact with it and therefore the secretory depressant in intestinal extracts is not this specific chalone, or (c) a complex chalone capable of inhibiting both secretion and motility has been fractionated by the extraction process and only the secretory depressing moiety has been recovered.

#### SUMMARY AND CONCLUSIONS

Fat in the intestine inhibits gastric motility induced by distention in both the vagally innervated and vagally denervated stomach. Intravenously administered urogastrone and "enterogastrone" extracts inhibit the distention-induced motility of the vagally innervated stomach but either have no effect upon or stimulate motility in the vagally denervated stomach.

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# CHRONIC AVITAMINOSIS E IN THE CASTRATE AND NON-CASTRATE RAT

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Evans and Burr (1) first demonstrated paralysis in the suckling young of vitamin E deficient rats. Olcott (2) examined the muscular system in these young paralyzed rats and discovered a remarkable widespread degeneration quite exactly resembling that reported by Goettsch and Pappenheimer (3) in their E low guinea pigs and rabbits.

The primary nervous involvement in avitaminosis E had been suggested by Einarson and Ringsted (4), emphasis being placed on the possibility of injury to the autonomic innervation of the musculature. The results of Pappenheimer and Goettsch (5) demonstrated the nervous origin of the muscular lesions when they found that the removal of nervous impulses by nerve section protected the muscles of young rats from the usual effect of vitamin E deficiency. Mason (6) proposes a primary myogenic origin of these lesions.

Consideration has been given to the involvement of the endocrine system in avitaminosis E and it has been generally believed that involvement of this system was purely secondary. This literature will be considered in the discussion of results.

**EXPERIMENTAL.** Six series of rats, 50 males to a set, were run.

1. A series on an unsupplemented vitamin E deficient diet. These rats was not castrated.

2. A series on a vitamin E deficient diet which received 5 mgm. of alphatocopherol daily. These rats were not castrated.

3. A series on an unsupplemented vitamin E deficient diet. This series was castrated before being placed on the diet.

4. A series on a vitamin E deficient diet which received 5 mgm. of alphatocopherol. These rats were castrated.

5. A series on a normal diet which were castrated.

6. A series of non-castrated rats on a normal stock diet.

The vitamin E deficient lard diet used throughout this investigation had the following composition:

Casein.....	27 per cent
Starch.....	35 per cent
Lard.....	22 per cent
Yeast.....	10 per cent
Salts.....	4 per cent
Cod liver oil.....	2 per cent

The cod liver oil is mixed into the ration at weekly intervals. It is omitted when the basic diet is made up. This is to prevent the destruction of vitamin A

by the rancid fat present in the diet. The possibility of destruction of vitamin A in this diet has been checked by intramuscular administration of vitamin A to the rats. As this treatment seemed to have no effect we have concluded that the diet is complete as regards the fat soluble vitamin A. The supplements of alpha-tocopherol were given by placing the material directly in the rat's mouth. Five milligrams of tocopherol were given in 0.25 ml. of corn oil.

The rats were weighed once each week. Symptomatically, they were under constant observation.

*Castrated rats on a normal stock diet.* These animals grow at a normal rate and at the end of a ten month period, attain a weight which is directly comparable with that of non-castrated rats on a normal diet. If these castrated rats are continued for varying periods they do gain weight but the gains caused by the accumulation of fat occur after twelve months. By the end of a fifteen month period, 5 per cent had attained a weight of 400 grams; all the others were either normal or but slightly increased in weight. A normal male rat on a normal diet will in fifteen months attain a weight of approximately 350 grams. There is therefore relatively little distinction even at this age. The impression prevalent that castration tends to cause an accumulation of fat has not been borne out in our observations on these animals. The 400 gram rats had a body which devoid of fur and outerlayer of body fat weighed 319 grams.

*Vitamin E deficient castrated rats.* Vitamin E deficiency in the castrated rat presents an entirely different picture. The animals were castrated shortly after weaning and placed on the lard diet. The course of growth is similar to that of a normal rat until six months have passed. Then, there is a sudden and marked increase in weight gains. At ten months, about 80 per cent are markedly obese. The fat is semi-fluid, not solid, and is present in such enormous quantities that the rat's body is completely hidden to the tips of the toes. The weights at the end of the ten month period reached from four hundred to six hundred grams. These weights were maintained throughout the entire experimental period, some fifteen months. All the non-castrated rats on vitamin E deficient diets had succumbed at 12 months. There is therefore an alteration in the course of a vitamin E deficiency in the absence of the testicle.

These rats when killed were dissected. The fur and the outer layer of body fat were removed which disclosed a body weighing only 150 to 250 grams. This means that in some instances the accumulated fat exceeded the weight of the body. The actual body weight was far below normal but exceeded that of the E deficient non-castrate rats at ten months. Three lines of evidence indicate the alleviation of the avitaminosis E in the absence of the testicle. First, the life span is prolonged. Second, the weight curve is better. Third, the accumulation of fat is marked.

*Vitamin E deficient non-castrated rats.* Vitamin E deficiency in the rat is characterized by retarded growth rate at four months and the development of muscular atrophy and paralysis at 8 to 10 months. This entire series of animals, fifty in number, all males, were placed on the lard diet at weaning. The observations of others were confirmed in that retardation of growth occurs and



muscular atrophy develops. The manifestations of this chronic E deficiency differ from those of other observers in that these rats showed a marked hunching of the back. This hunching, as is shown in figure 2, is profound; there is a curvature of fully seventy-five per cent of all the vertebrae of the spinal column. The scapulae due to a complete and total atrophy of the subscapularis muscles, are loose, they do not seem to be attached to the body. The digits of the paws are held in an obstetrical position, clinched with the digit corresponding to the thumb turned under and held by the other digits. These animals if placed on their sides cannot right themselves. The paralysis is entirely posterior and not

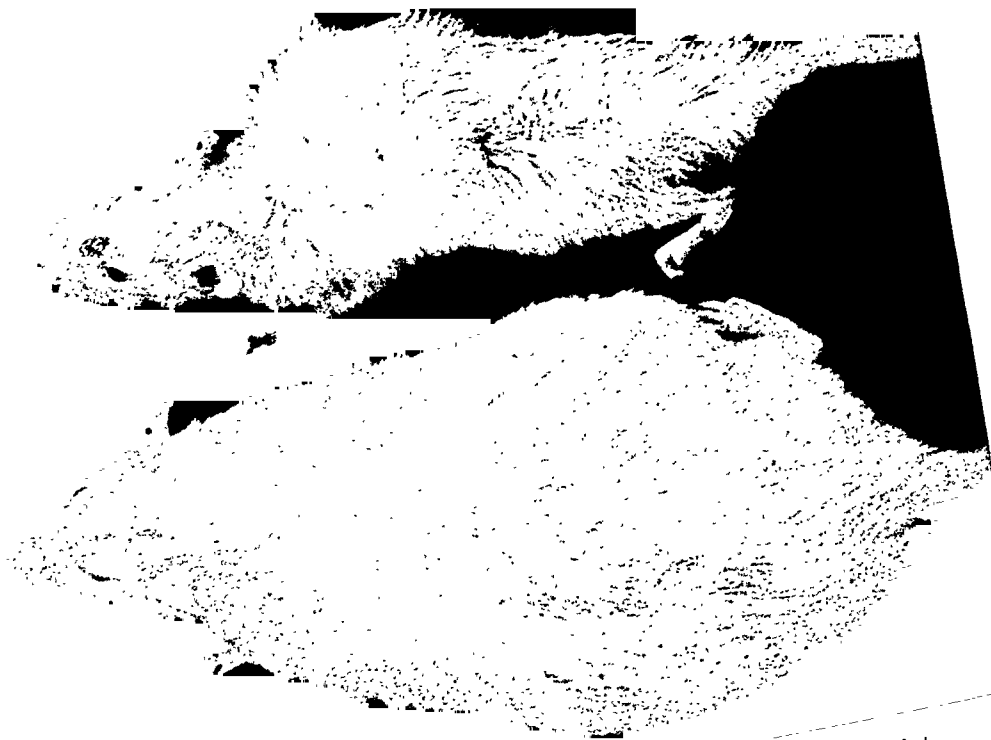


Fig. 1. Non-castrate and castrate rats on vitamin E deficient diet.  
Upper: Non-castrate, E deficient rat.  
Lower: Castrate, E deficient rat.

complete. Under stress, the animal will voluntarily move his hind legs. The fur of these animals is rough and unkempt, occasionally a mild form of alopecia is seen. The nose is sometimes crusted and wet appearing. The weight attained by these animals in ten months is from 100 to 150 grams. One hundred per cent of rats will develop these symptoms if kept on the diet long enough. The entire series died between the 10th and 12th months.

*Radiographic findings in avitaminosis E rats.* The scapula is no longer adherent to the thoracic cage. The obstetrical position of the forefoot is interesting. The most striking feature is the marked kyphosis, which is in the thoracic

region and does not occur elsewhere. There is a sharp angulation at the point of attachment of the 12th rib and again at the point of attachment of the 1st rib, in the cervical thorax. Throughout the thoracic region, the spinal column is perfectly straight. The respiratory movement of these animals is more rapid and the excursions of the thorax are deeper. The attempts to maintain adequate respiration may bring about the kyphosis much as the mechanism which causes kyphosis in the emphysematous patient.

Castrated rats on a vitamin E deficient diet supplemented by tocopherol were identical in all gross respects with the castrated rats on a normal stock diet.

The other two series of rats, namely, the normal rats on normal diets and the normal rats on the vitamin E deficient diet supplemented by 5 mgm. of alpha-tocopherol, grew entirely normally. Figure 3 shows the growth curves for all six series.

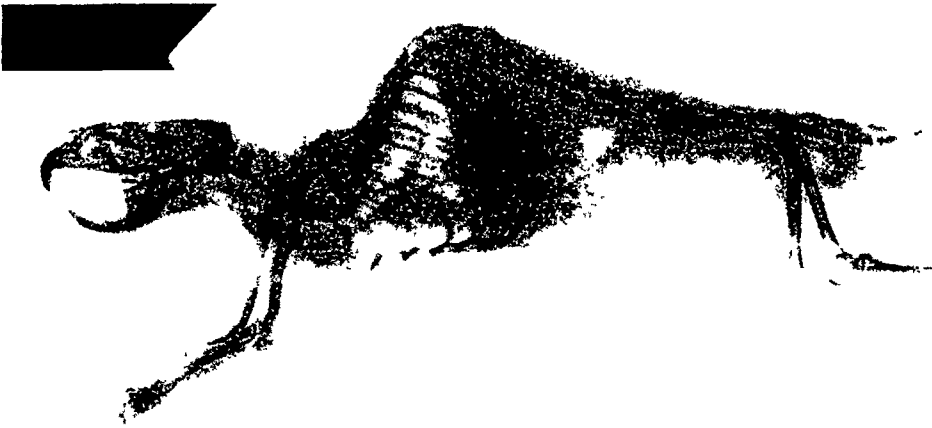


Fig. 2. Radiograph. Non-castrated rat, vitamin E deficient

DISCUSSION. Biddulph and Meyer (7) carefully studied the effects of avitaminosis E on endocrines. The testis degenerate to a weight of one-half normal, accessory sex glands increase in weight for three months and then decrease. Adrenal weights in males increased, no change in the female. Involution of thymus was delayed in the male but not in the female. Thyroids of males increased as much as 100 per cent in weight, no change in female. The pituitaries of males showed an increase of basophils. Females showed no change. They noted an increase in luteinizing hormone content of the pituitary glands of vitamin E deficient males when compared with normals. This effect was not as great as that seen in castrate males. Van Wagenen in 1925 (8) and Nelson in 1933 (9) had previously reported on the castration changes occurring in the anterior pituitary glands of vitamin E deficient rats.

The histopathology of the testis in avitaminosis E is too well known to warrant reviewing here. The germinal epithelium is irreversibly degenerated. The Sertoli cells remain. It is the generally accepted view that the interstitial cells of testes of mammals are the source of androgen. Testosterone brings about sper-

matogenesis by stimulating the pituitary to secrete gonadotropic hormones (10). There seems to be no report of the testosterone content of testicles of an avitaminotic rat. Adamstone (11) reported that caponized E deficient male fowls responded much more rapidly to combined tocopherol and testosterone than to testosterone alone. Bomskov and Kaulla (12) found normal hormone production in the ovary and testicle of rats on a vitamin E deficient diet.

The chronic vitamin E deficient animal has no adipose tissue despite high fat content of diet (13). Some forms of obesity are due to a hypothalamic disorder,

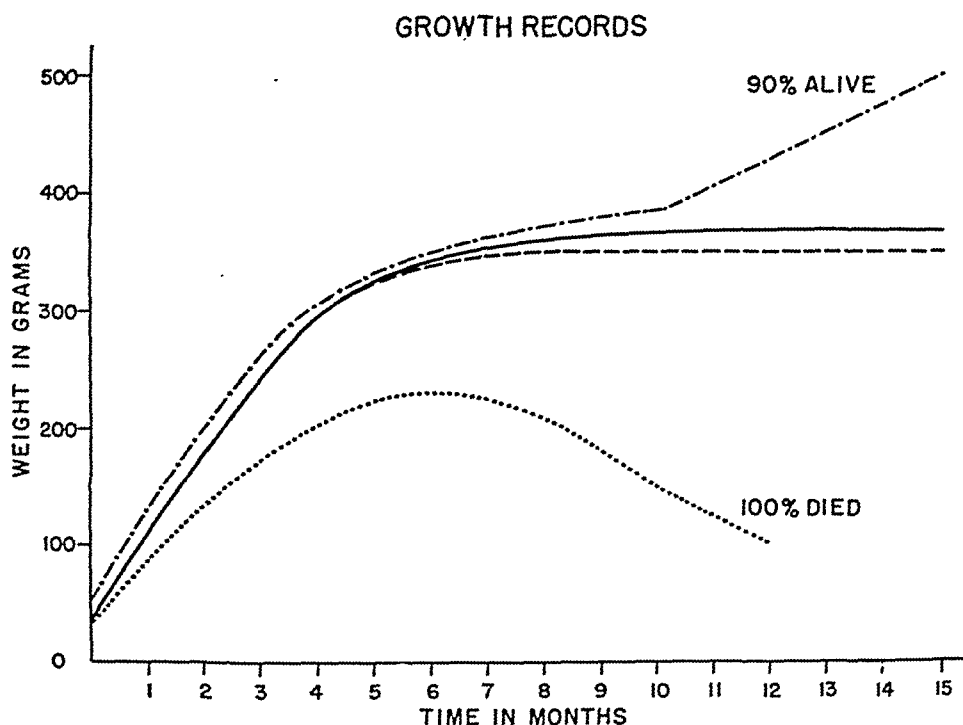


Fig. 3. Growth curves of avitaminosis E castrate and non-castrate rats

- ..... Normal rats, E deficient diet
- .... Castrated rats, E deficient diet,
- Castrated rats, normal diet
- Castrated rats, E deficient diet,
- tocopherol supplemented
- Normal rats, E deficient diet,
- tocopherol supplemented
- normal rats, normal stock diet

to disease of the pituitary, of the adrenal cortex or to hypofunction of the gonads. These are the endogenous varieties of obesity. Gross size changes have not been found in castrated rats and guinea pigs (14), and whether consistent results are obtainable for any mammal is yet to be demonstrated. Korenchevsky (15) concluded that gonadectomy leads to a lean castrate in 40 per cent of cases and to a fat castrate in 60 per cent; however, Commins (16) concludes that early castration in rats exerts a retarding effect upon weight accumulation. A sixteen month castrate dog (17) rated a fat castrate was found to have gained less weight than a control normal female under identical conditions.

In our experiments normal castrated animals did not become obese. Top weight in the series was 450 grams. Therefore our experiments confirm the general conclusion that in the rat, castration does not lead to obesity. Animals on the vitamin E deficient diet supplemented with alpha-tocopherol were normal in every gross respect, whether castrated or not. These various series control the findings in the E deficient castrates and non-castrates. Avitaminosis E in the non-castrate animal results in a profound dystrophy and terminal paralysis. Interpretation of our results is difficult. Castrated vitamin E deficient rats survive for greater periods of time than do non-castrated vitamin E deficient animals. The effect of avitaminosis E on fat metabolism is entirely reversed in a castrate.

#### SUMMARY

Chronic avitaminosis E symptomatically runs a markedly different course in the castrate and the non-castrate rat. Three lines of evidence are reported indicating the effect of castration on this vitamin deficiency state. First, the life span is prolonged. Second, the weight curve is improved. Third, there is a marked accumulation of fat.

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# EXPERIMENTAL STUDY OF ANTIPERISTALTIC AND PERISTALTIC MOTOR AND INHIBITORY PHENOMENA<sup>1</sup>

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In this paper a new, simple method will be described that permits the direct observation of peristalsis and antiperistalsis with a minimum of manipulation and instrumentation. The site is the descending colon of a barbitalized rabbit and the effective stimulus is a normal, dry rabbit scybalum. Experimental evidence will be submitted that peristalsis and antiperistalsis are fundamentally alike in their motor and inhibitory manifestations and that in both a wave of inhibition precedes the wave of contraction. Our results are thus in basic accord with the law of the intestine first described by Bayliss and Starling for peristalsis (1a, 1b, 1c) and we extend its application to antiperistalsis, about which Bayliss and Starling say nothing. Among other facts, we shall also present new experimental evidence that a reciprocal relation exists between the longitudinal and circular muscle layers so that when one contracts, the other relaxes.

It will be remembered that a number of investigators, especially Alvarez (2) have been unable to demonstrate any definite relaxation or inhibition of the gut preceding a moving bolus; we, on the other hand, believe that our experimental work has laid bare sound evidence that such inhibition does occur.

**TECHNIQUE.** Rabbits only were used; their weight ranged from 2.5 to 3.5 kgm.; the majority were males and their food was rabbit-chow pellets with water ad libitum; they were narcotized by the subcutaneous injection of 150 mgm. of sodium barbital per kgm. After 30 minutes, the descending colon was fully exposed through a midline incision, the cecum, ascending colon and small intestines being displaced into folds of wet towel. The thin ligament connecting the descending colon to the ascending loop of the duodenum was cut and 10 cm. of descending colon were available for easy observation. About 2 cm. above the pelvic brim, the descending colon was cut through 60 per cent to 80 per cent of its circumference opposite to the mesenteric border (aboral cut); a second cut of the same size was made 6 to 8 cm. oral to the first cut. Then a dry normal rabbit scybalum was pushed into the oral end of the segment and a peristaltic wave resulted sooner or later. Blocking the progress of this scybalum, brought about shortly a contraction in the digitally compressed area and this contraction travelled in an antiperistaltic direction. Thus both peristalsis and antiperistalsis could be easily studied in one fixed area.

The moderate use of physostigmine is desirable in general, particularly if the

<sup>1</sup>A preliminary note was published in the *Proc. Soc. Exper. Biol. and Medicine* 57: 360, 1944.

segment is sluggish; the necessary subcutaneous dose varies from 0.1 to 0.3 mgm. of the sulfate per kgm.

**EXPERIMENTAL RESULTS.** No definite motility of the descending colon may occur after exposure even though it is crammed with well-formed, dry scybala. After placement of the two cuts, both peristalsis and antiperistalsis may be observed, but clear analysis is not possible until both the segment and adjoining sections of the descending colon have been cleared of scybala. Now a dry normal scybalum is pushed 1 to 2 cm. into the oral end of the segment. This produces a contraction of the circularis muscle layer oral to the scybalum, and the scybalum begins to move peristaltically towards the aboral cut. If the progress of the scybalum is now blocked by gentle, digital compression, a series of peristaltic contractions and relaxations develop that become successively longer and stronger, until the gut oral to the blocked scybalum blanches to a gray-white, bloodless cord by a tetanic contraction of the circularis layer, but the section compressed by the fingers remains soft, pink-red and relaxed. Suddenly, as the last peristaltic contraction wave begins to relax and turn pink, one feels that the compressed relaxed area now contracts and on removal of the fingers, this contraction of the circularis layer travels antiperistaltically with the scybalum in front of it.

The new antiperistaltic wave of contraction appears as a gray, narrow cord with transverse, fine corrugations spaced a fraction of a millimeter apart; its length during travel remains practically constant but varies at different times and in different waves from 0.5 to more than 1 cm. A peristaltic wave of contraction is similar to an antiperistaltic one and in both one may often observe that the gray, tetanically contracted cord of circularis muscle is bordered by a faint, thin red line (fig. 1).

Careful examination of the aboral side of a scybalum advancing antiperistaltically reveals that the colonic wall fits snugly over the scybalum and is thrown into 3 or 4 transverse ridges about 1 mm. apart (fig. 1). During travel of the scybalum, those ridges pass down the aboral surface, contracting gradually until they become the advancing border of the pale, antiperistaltic contraction-wave. As those ridges become part of the contraction-wave, the other, aboral, end of the contraction-wave begins to relax and turn pink, so that the contraction-wave remains practically constant in length. In a peristaltic wave of contraction the same effects are observed in a reverse direction.

However on the oral side of a scybalum advancing antiperistaltically, conditions are quite different. On this side, the colonic wall fits only loosely over the scybalum, the color is pink-red with easily visible venules, there are longitudinal folds and this section feels soft on palpation (fig. 1). This relaxed section is not stationary, but precedes the traveling scybalum apparently by 1 to 3 cm.; the colonic wall in this state does not respond to moderate stimuli. If the stimulus is too strong or if the antiperistaltic progress of the scybalum is blocked by digital compression, then events take place that are similar to those described above, except that now a peristaltic wave is produced.

Now and then a scybalum traveling either peristaltically or antiperistaltically

in the segment, slowed, stopped and its wave of contraction relaxed and faded away without any sign of an opposing contraction in the segment. Careful inspection soon revealed that two scybala were approaching each other on opposite sides of the cut; when this happens one or the other scybalum becomes immobilized while the other keeps on moving before its wave of contraction. For example, a scybalum moving antiperistaltically in the segment, stops and its wave of contraction relaxes, while another scybalum moving peristaltically in the colon above the oral cut keeps on moving towards this cut. This second scybalum may now be expelled at the cut, but its wave of contraction passes through the cut into the segment and then moves the quiescent scybalum in a peristaltic direction through the segment. At other times, the antiperistaltic wave in the segment may prevail and its scybalum is expelled at the oral cut

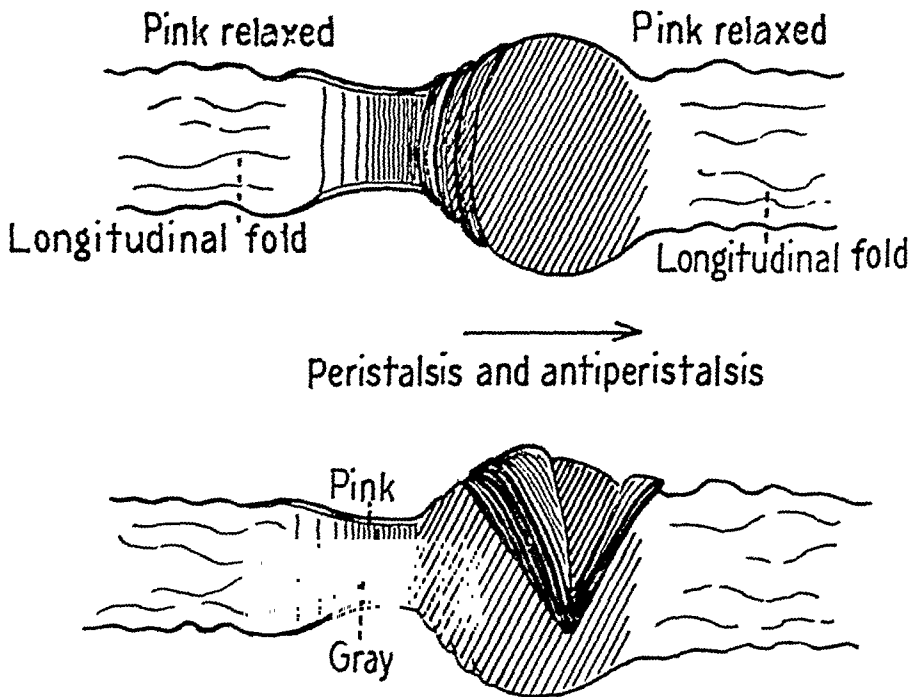


Fig. 1. Schematic; for explanation see labeling and text

while the scybalum above the cut remains motionless because its peristaltic wave of contraction relaxed.

Similar effects are readily seen at the aboral cut when a peristaltic contraction-wave traverses the segment while an antiperistaltic contraction-wave and its scybalum in the pelvic colon is approaching the same cut. It therefore appears that the progress of a scybalum in the segment is influenced by motor activity of those parts of the colon that are adjacent to the segment.

At each cut, two lips are formed, one oral, the other aboral, and both show a narrow eversion that is about 1 mm. wide. This eversion is composed of the entire wall of the colon, the mucosal surface being external. When a scybalum traveling antiperistaltically in the segment, begins to enter the oral cut, the slight eversion of the aboral lip disappears and the lip thins out as it passes over the

oral surface of the scybalum; but as it passes down the aboral surface of the scybalum, this lip is now so strongly everted that it measures 3 to 4 mm. (fig. 1). The other or oral lip is well relaxed, pink, shows but little eversion and its adjacent colon is also relaxed; into this relaxed area the scybalum now enters, if the two lips are moderately aligned. After complete entry, a powerful traveling wave of contraction appears aboral to this scybalum, and the scybalum moves antiperistaltically for a variable distance. The lip however through which the scybalum passed last shows but little eversion. There is thus a marked difference in the appearance of the two lips after complete transit of the scybalum. The same difference will be seen if an antiperistaltic contraction-wave proceeds from the pelvic colon to the segment, or if scybala pass the oral and aboral cuts peristaltically: always the first lip traversed by the scybalum shows a strong eversion of the gut-wall while the other lip shows relatively little curling (fig. 1).

Peristaltic and antiperistaltic contraction-waves pass through the 60 per cent to 80 per cent cuts as smoothly as if the descending colon were intact. If the entire gut is severed, without interfering with the blood supply, marked shortening of the segment must be prevented by a suture at the mesenteric border of each cut. Under these conditions, a 100 per cent cut prevented the passage of contraction-waves except in a few doubtful instances and the passage of inhibitory waves was never seen. It must be recorded that relatively few experiments were made with 100 per cent cuts.

The speed of an antiperistaltic contraction-wave was always much slower than that of a peristaltic contraction-wave. For example, 3 different antiperistaltic waves moved the scybalum 1 cm. in 22.5, 20 and 16 seconds, while 4 peristaltic waves moved the scybalum 1 cm. in 3.5, 4.3, 4.8 and 3.5 seconds. These speeds were observed in fully innervated colon-segments after the injection of physostigmine.

The speed of the relaxation-wave could not be determined with any degree of accuracy.

Influences arising in the central nervous system were excluded from the segment by transection and pithing of the cord from the 10-12 thoracic level and in addition the vagi and splanchnic nerves were cut below the diaphragm. In 10 experiments of this type, antiperistalsis was seen in 2 rabbits only and in one of these 2 it occurred only once; the other 8 rabbits showed no antiperistalsis. This absence of antiperistalsis occurred in rabbits with and without physostigmine. Peristalsis however was present in all 10 animals.

In 15 experiments with central innervation intact, 11 showed antiperistalsis in the segment; in 4 it was slight. Peristalsis was seen in 14; in one, no peristalsis was observed.

In a number of physostigminized rabbits, the empty segment showed 3 to 4 funnel-shaped, bloodless constrictions that were separated by slightly dilated, definitely pink areas, the pink areas being formed by the junction of wider parts of two adjacent funnels. These constrictions formed gray-white cords 1 to 2 cm. long and the intervening moderately relaxed areas were pink, apparently empty and about 0.25 cm. long.



In another type, the constrictions were just as powerful but measured only 0.5 cm. in length while the dilated sections were 1 to 2 cm. long; the dilatations were filled with gas.

These types of multiple segmental contractions and relaxations occurred simultaneously, spontaneously, showed no evidence of travel during observation and were present with and without central innervation.

*Interpretation of Experimental Data. Evidence for existence of inhibition.* The chief experimental data that a traveling wave of inhibition precedes a traveling wave of contraction, both in antiperistalsis and peristalsis, are the following observations.

a. In a highly irritable segment of the colon, a scybalum may lie motionless between two bloodless cords of constricted circularis muscle. Sooner or later one of these constrictions turns pink and then relaxes visibly, while the other constriction shows no immediate change; then the scybalum begins to move into the relaxed area as the sustained constriction becomes a traveling contraction-wave; the area of relaxation also progresses at the same time and precedes the scybalum as long as the contraction-wave travels on its way. Here obviously the scybalum was not driven by the brute force of one contraction into another area of contraction, for no movement of the scybalum occurred until one constriction began to relax. This blushing on one side of the scybalum is the first sign of relaxation for it heralds the entry of blood into the previously bloodless area, and this blushing is then followed by a visible relaxation of the constricted area. The direction of movement is always into the relaxed area and may be peristaltic or antiperistaltic.

b. In a normally irritable segment, a moving scybalum shows on one side a few millimeters of contracted, pale circularis muscle while the colon on the other side of the scybalum is pink and relaxed with longitudinal folds (fig. 1). This relaxed, longitudinally folded area is moderately refractory to stroking stimuli, thus indicating inhibition.

c. The progress of a scybalum moving either peristaltically or antiperistaltically in the segment, may be influenced by motor activity in the colon adjacent to the cuts. Thus if two scybala on opposite sides of a cut are approaching each other, one or the other of these scybala slows, stops and its wave of contraction relaxes, while the other scybalum with its wave of contraction progresses and expels its scybalum at the cut. This process is observable both at the oral and aboral cuts; in all instances, one scybalum with its contraction-wave moves towards the relaxed area and it is immaterial whether this movement is peristaltic or antiperistaltic. These facts can only be explained by the assumption that an inhibitory wave precedes the wave of contraction, and the stronger inhibitory wave of the two inhibits the contraction-wave of the other.

These experimental findings are strong evidence that a traveling wave of inhibition precedes a traveling wave of contraction both in peristalsis and in antiperistalsis; that inhibitory and contraction waves are definitely linked together to form a unit; and finally that inhibition may exist even between apparently stationary areas of contraction.

*Functional relation of circular and longitudinal muscle layers.* Our experimental

procedure supplies evidence that when one muscle layer contracts, the other adjacent muscle layer relaxes. On earlier pages we described the strong eversion of the first lip of a cut that occurs when the scybalum is passing (fig. 1) or has passed through a cut. This eversion of the circularis muscle layer with its sub-mucosa and mucosa could only be produced by a contraction of the longitudinal muscles at a time when the circularis layer was inhibited, for otherwise it would be impossible for the thin, weak longitudinal layer to evert the thick layer of circularis muscle. That the circularis is inhibited is shown by the ease with which it thins out into a ribbon during its transit over the widest part of the scybalum and during this transit one can see the eversion taking place, forming a lip 3 to 4 mm. wide.

This contraction of the longitudinal muscles with accompanying relaxation of the underlying circularis layer is also seen during the passage of a scybalum through the intact section of the segment-tube. Here again one notes not only that the circularis muscle layer becomes ribbon-like as it passes over the scybalum, but also that the relaxed circularis layer is drawn together by a contraction of the longitudinal muscle layer and practically lifted over the rough surface of the scybalum. Furthermore, as these grouped, relaxed, circularis fibers pass down the other face of the scybalum, an interesting morphological change appears that has not been described so far: the grouped circularis fibers contract and form several easily visible, transverse ridges (fig. 1). These ridges are about one millimeter apart and these intervals must be attributed to relaxation of the longitudinal muscle fibers during contraction of the circularis layer. In this one example we see contraction of the longitudinal muscles accompanied by relaxation of the circularis layer, and contraction of the circularis layer accompanied by relaxation of the longitudinal layer.

Another piece of evidence that contraction of the circularis layer is accompanied by relaxation of the longitudinal layer, is the observation that a traveling wave of contraction is often bordered by a thin, pink line (fig. 1). This pink line is apparently the blood-containing, relaxed longitudinal muscle layer.

*Length of the local inhibitory wave.* The local inhibition of the circularis layer accompanying contraction of the longitudinal muscle layer, appears to be about 4 millimeters. This is indicated by the width of the lip everted by contraction of the longitudinal muscle fibers (fig. 1).

*Mediation of impulses between the circular and longitudinal muscle layers.* The passage of either motor or inhibitory influences between the two muscle layers must take place largely through the muscle cells themselves, for relatively few of the smooth-muscle cells are provided with nerve endings (3) and in fact, the muscle cells of the two layers are structurally closely related. In tangential sections of a dilated, descending colon of rabbit we have seen muscle cells pass at various places from the circular layer into the longitudinal layer and vice versa, while the separate muscle ribbons of the circularis are connected to each other by slender muscle-strands that are 2 to 5 muscle cells in thickness; these strands pass from 8 to 2 o'clock or from 4 to 10 o'clock. Interlacement of the two muscle layers has also been described in dog (4).

*Direction of travel of an unseen contraction wave.* We have already described

that the first lip of a cut passed by a scybalum and its contraction wave is strongly everted while the second lip shows but little eversion (fig. 1). Since this difference persists for some time in the empty condition of the colon, and since this difference occurs both in peristalsis and antiperistalsis, mere inspection of a cut often betrays in what direction an unseen contraction wave has passed.

*Differences between antiperistalsis and peristalsis.* Antiperistalsis is almost entirely abolished by removal of influences from the central nervous system while peristaltic waves of contraction and inhibition remain apparently unimpaired; this was observed whether or not physostigmine had been injected. Another difference is seen in the speed of travel: the antiperistaltic wave of contraction is usually much slower than that of a peristaltic wave, the ratio being roughly 1:4. And thirdly, the strength of contraction of an antiperistaltic wave in the segment appeared usually to be definitely less than that of a peristaltic wave.

The failure of Bayliss and Starling (loc. cit.) to observe antiperistalsis in their extensive studies is attributable to the routine pithing of the cord in their experimental material.

#### SUMMARY

Partial isolation of a segment of the descending colon in a barbitalized rabbit, readily permits the study of peristalsis and antiperistalsis by mere inspection.

Peristalsis is produced by inserting a dry, normal scybalum into the oral end of the empty colonic segment.

Antiperistalsis is produced by temporarily blocking a peristaltic wave of contraction in the segment until an antiperistaltic contraction wave appears.

The antiperistaltic and peristaltic waves of contraction are preceded by a demonstrable wave of inhibition and the inhibited area is relatively refractory to moderate stimuli.

The antiperistaltic and peristaltic waves of contraction and inhibition pass the partial cuts in the colon without any obvious pause.

If the cuts sever the entire circumference, the contraction waves do not pass through except in a few doubtful instances. Inhibitory waves were not seen crossing a 100 per cent cut.

Removal of influences from the central nervous system practically abolishes antiperistalsis; typical peristalsis however persists.

The speed of an antiperistaltic wave of contraction is considerably slower than that of a peristaltic wave of contraction.

New evidence is presented that contraction of the longitudinal muscle layer is accompanied by an inhibition of the underlying circularis fibers, and conversely, a contraction of the circular layer is accompanied by a relaxation of the longitudinal muscle layer.

The formation of transverse ridges in the colonic wall covering that face of a scybalum adjacent to the wave of contraction, is explained by this reciprocal relation of the two muscle layers.

It can easily be shown in tangential sections of the dilated descending colon

that muscle fibers of the circular layer pass directly into the longitudinal layer and vice versa.

Inspection of the two lips of a cut may betray whether an unseen contraction-wave was peristaltic or antiperistaltic; the first lip passed by the scybalum is more strongly everted than the second lip.

The experimental procedure used in this work is simple, efficient and new.

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# THE EFFECT OF METHIONINE ON PROTEIN-DEFICIENT RATS EXPOSED TO BENZENE<sup>1</sup>

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In previous communications (1, 2) it was demonstrated that the protein content of the diet is of primary importance in determining the susceptibility of rats and dogs to benzene poisoning and that exposure to benzene, furthermore, retards the growth of rats to which a diet low in protein is fed. Leukopenia occurred among a larger percentage of the animals which had been exposed to benzene while partaking of a diet that was deficient in protein, and was more severe among them than it was among animals which had been exposed to benzene while partaking of an adequate diet. It is well established that conjugation as the sulfate is important in detoxication of benzene (1, 3). The sulfur compounds which can be used for this purpose have been the subject of several investigations (4-8). Normally, protein is the principal source of sulfur in the diet. Consequently, the intake of sulfur is a factor that may influence the susceptibility to benzene of an animal that is deficient in protein.

An experiment was planned to determine the effect of methionine on the toxicity of benzene for growing rats that were being fed a diet containing 9 per cent of casein. It was believed that if methionine were fed in excess of the amount required for growth, excess sulfur should be available for detoxication. An excess of methionine, it was thought, would eliminate sulfur as a factor in any restriction of growth that might occur upon exposure to benzene. The experiment was as follows:

**PROCEDURE.** Four groups of male albino rats which weighed about 100 grams each were fed an adequate basic diet for two weeks. Then the diet of the rats of all groups was changed to one that was low in protein and the diet of two groups was supplemented with sufficient methionine to make the intake of sulfur equal to that provided by a diet which contains 30 per cent of casein, calculated according to Kassell and Brand's (9) analysis. The composition of these rations is shown in table 1. One group which received the basic diet and one which received the supplemented diet was exposed forty hours weekly for twelve weeks to an atmosphere containing 600 parts per million by volume of commercial benzene (90 per cent  $C_6H_6$ ), as previously described (1). The other two groups, one of which received the basic diet and one the diet supplemented with methionine, were kept in chambers similar to those in which were kept the rats that were exposed to benzene. The same amount of air circulated

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through all of the chambers, and all of the rats which completed the experiment were confined for the same length of time.

Animals were housed in individual cages with false bottoms. Any rat which acquired an infection of the respiratory tract was discarded. Water and food were provided so that they could be taken at will but the consumption of food was measured carefully. All animals were weighed twice a week. Leukocyte counts of the blood were made as follows: (1) of three rats from each group at weekly intervals and (2) of all rats which were exposed to benzene at weekly intervals for the last five weeks. The concentration of hemoglobin in the blood of three animals from each group was determined weekly by Wu's (10) method. All rats were killed at the end of the twelfth week of the experiment and necropsy was performed. The livers of individual rats were weighed. Next, the livers

TABLE 1  
*Composition of the diet*

	BASIC DIET	METHIONINE DIET
	<i>per cent</i>	<i>per cent</i>
Casein (Labco).....	9	9
Methionine.....		0.76
Lard.....	15	15
Sucrose.....	69	68.24
Wesson's salt mixture (16).....	4	4
Cellophane.....	3	3

Two drops cod liver oil and 500 mgm. brewers' dried yeast per rat daily.

TABLE 2  
*Gain in weight*

EXPOSURE	DIET	RATS, NUM-BER	AV. MAX. WT. AT-TAINED	AV. GAIN
			<i>gram</i>	<i>gram</i>
Benzene	Basic	9	190.6	10.9 $\pm$ 15.9
	Methionine	7	273.4	87.4 $\pm$ 18.4
Air	Basic	8	229.5	53.0 $\pm$ 7.3
	Methionine	6	315.9	129.0 $\pm$ 10.2

1. Difference between two groups exposed to benzene, 76.5.
2. Difference between two groups fed basic diet, 42.1.
3. Difference between two groups exposed to air, 76.0.
4. Difference between two groups fed methionine, 41.6.

of the rats of each group were pooled and were analyzed for total lipids, according to the method of Best, Channon and Ridout (11).

RESULTS. *Growth.* The average curves of growth for the four groups are presented in figure 1. The average total gain in weight and the average maximal weight attained are recorded in table 2. Rats of all groups initially lost weight when they were given the diet low in protein but they grew at varying rates thereafter. Those exposed to benzene lost more weight at the beginning and resumed growth later than the rats of corresponding groups which were exposed only to air. The rats which received the diet that was supplemented with methionine lost less weight, and started to gain again sooner, than the corresponding animals which received the basic diet. Animals of the group which received the basic diet and were exposed to benzene lost as much as 34 grams and barely had regained their initial weight by the end of the experiment.

Exposure to 600 parts per million of benzene further retarded the growth of rats fed a low protein diet. Rats exposed to benzene, and fed methionine, gained 76.5 grams more than the rats which received the unsupplemented diet and were exposed to benzene. The rats exposed only to air and fed methionine gained 76.0 grams more than the rats which received the unsupplemented diet and were exposed only to air. The difference between the exposed and unexposed groups fed the basic diet is 42.1 grams and the difference between the two groups fed methionine is 41.6 grams. Feeding of additional methionine promoted growth of rats which were deficient in protein, whether they were exposed to

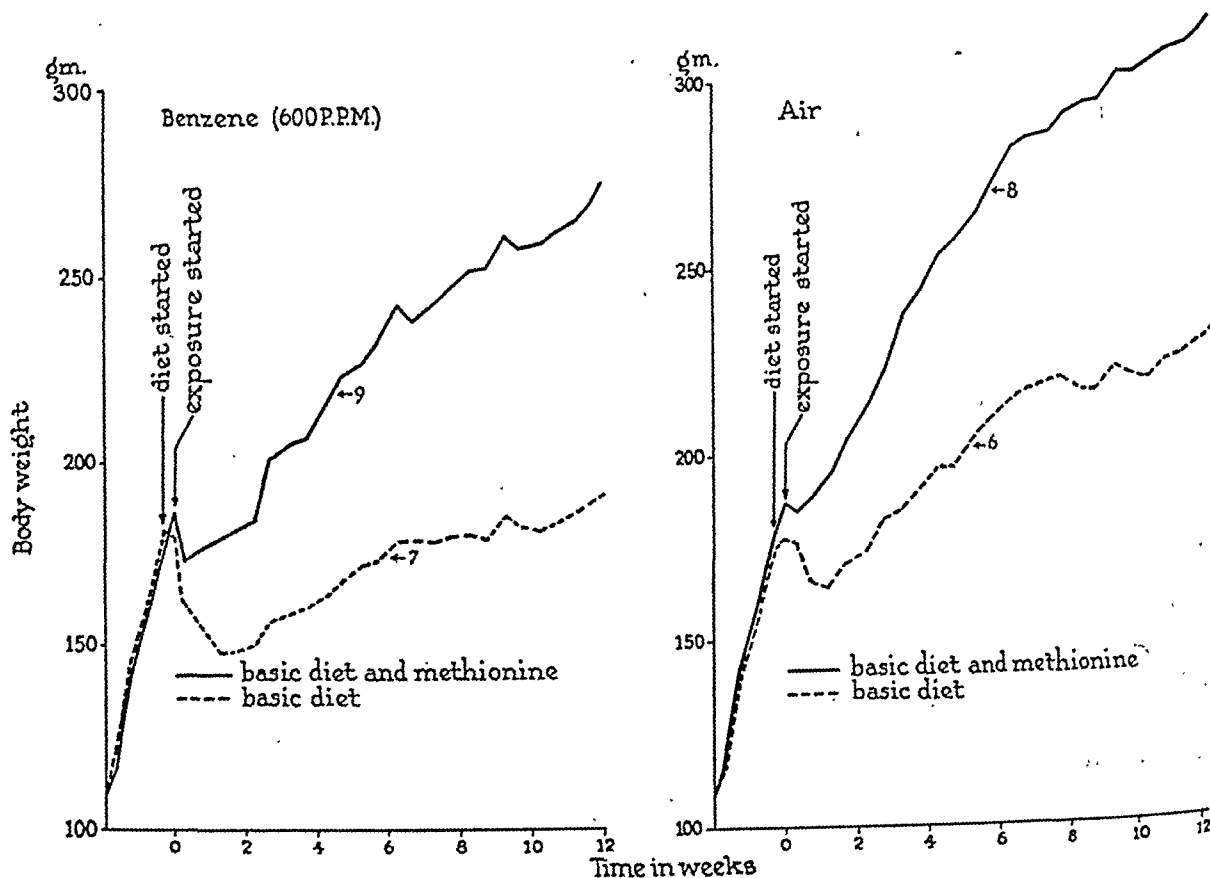


Fig. 1. Curves of growth. Numbers near the curves indicate rats included

benzene or only to air. However, rats which were exposed to benzene and were fed methionine were definitely smaller than rats which were fed methionine and were exposed only to air.

*Leukocytes.* Averages of the weekly leukocyte counts made on the blood in the last three weeks of the experiment, and the incidence of leukocyte counts of less than 10,000 per cubic millimeter obtained in that same period, are given in table 3. In figure 2, the individual counts covering the whole period of the experiment are represented. Although the counts varied widely, those made on the blood of animals which were exposed only to air were definitely higher. Animals of both groups which were exposed to benzene manifested leukopenia

but the decrease in the number of leukocytes occurred earlier and was more marked in the group which received the basic diet only.

The difference between the number of leukocytes present in the blood of animals which were exposed to benzene and received the basic diet on the one

TABLE 3  
*Leukocytes (per cubic millimeter of blood)*

EXPOSURE	DIET	RATS, NUMBER	FINAL WEEK	AV. OF LAST 3 WEEKS	LESS THAN 10,000 IN LAST 3 WEEKS, INCIDENCE
Benzene	Basic	9	11,000	10,400 $\pm$ 3,100	12
	Methionine	7	13,500	13,900 $\pm$ 2,500	4
Air	Basic	3	22,100	23,000 $\pm$ 5,500	0
	Methionine	3	17,400	19,000 $\pm$ 1,500	0

TABLE 4  
*Hemoglobin in last three weeks (grams per 100 cc. blood)*

EXPOSURE	DIET	RATS, NUMBER	HEMOGLOBIN
Benzene	Basic	3	13.3
	Methionine	3	14.6
Air	Basic	3	12.9
	Methionine	3	14.2

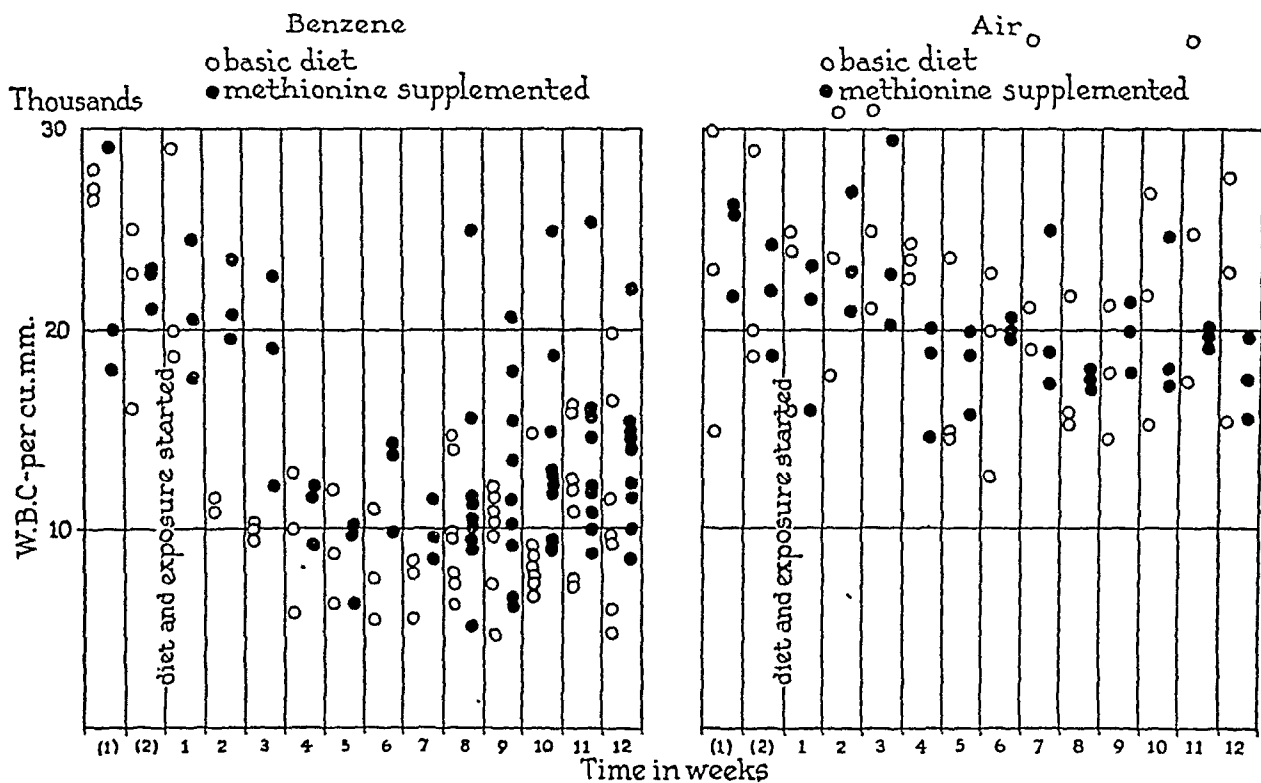


Fig. 2. Leukocyte counts

hand, and on the other, in the blood of those which, although they also were exposed to benzene, received the supplemented diet, was on the verge of being significant (the critical ratio  $C_1 = 1.9$ ). In the last three weeks of the experiment, the incidence of leukocyte counts of less than 10,000 per cubic millimeter of blood was less among animals which were exposed to benzene and were fed



methionine than among those which were exposed to benzene and were not fed methionine.

*Hemoglobin.* Animals of all groups had some degree of anemia. Averages of the last three determinations of concentration of hemoglobin in the blood are found in table 4. The blood of animals of both groups which received methionine gave higher values for hemoglobin than animals which received basic diets. Exposure to benzene had little or no effect on the concentration of hemoglobin.

*Intake of food.* The average intake of food in the last three weeks of the experiment is given in table 5. The animals ate less when exposed to benzene than when exposed to air. More food was consumed when it was supplemented with methionine than when it was not so supplemented. As would be expected, the caloric intake per unit of body weight was greater among the rats which received the basic diet, since the animals were of smaller size.

*Analysis of liver.* In table 6, the average weight of wet livers from animals of various groups and the lipid content of the pooled samples are represented.

TABLE 5

*Intake of food in last three weeks (per day per rat)*

EXPOSURE	DIET	RATS, NUMBER	FOOD, GRAMS	CALORIES PER 100 GRAMS RAT
Benzene	Basic	7	8.6	20.7
	Methionine	9	11.5	19.7
Air	Basic	6	10.3	20.5
	Methionine	8	13.0	18.9

TABLE 6

*Analysis of liver*

EXPOSURE	DIET	RATS, NUMBER	WET WT., GRAMS	TOTAL LIPIDS		
				Conc., per cent	Total amt., grams	Per 100 grams rat, grams
Benzene	Basic	7	12.7	28.5	3.62	1.90
	Methionine	9	11.1	7.0	0.78	0.28
Air	Basic	6	15.6	34.5	5.38	2.35
	Methionine	8	13.1	8.2	1.07	0.34

Animals which received the basic diet and which were exposed only to air had the largest livers (average, 15.6 grams) and the concentration of total lipids in their livers was highest (34.5 per cent). Animals which received the basic ration and were exposed to benzene failed to grow and their livers contained less fat, as compared with animals which received this same ration but were exposed to air only. Regardless of whether or not animals were exposed to benzene, if they received diets supplemented with methionine they had smaller livers, of lower fat content, than animals which received the basic diet only.

COMMENT. The effect of methionine on the growth of rats which are maintained on a diet containing 9 per cent of casein is striking. According to Treadwell (12), the rat requires for growth 600 mgm. of methionine per 100 grams of diet and another 600 mgm. of methionine per 100 grams of diet for its lipotropic effect when the diet contains 40 per cent of fat. By calculation, it appears that the diet which Treadwell employed supplied 186 mgm. of methionine per 100 calories. The diet used in the experiment here reported supplied 235 mgm. of methionine per 100 calories. The rate of growth of the rats which received

a supplemented diet and which were exposed only to air was similar to that of rats maintained on a diet which contained 30 per cent of casein (2). Glynn, Himsworth and Neuberger (13) found that rats grew rapidly while they were being fed a mixture of amino acids that was free of sulfur and that was supplemented by large amounts of methionine. Miller (14), working with dogs which were maintained on a diet low in protein, demonstrated that methionine and cystine had a protein-sparing action.

The growth-increasing effect of methionine on rats which were exposed to benzene (76.5 grams) as well as on their controls (76.0 grams) proved that the low intake of methionine was an important factor in restriction of growth of rats maintained on a diet which contained 9 per cent of casein. The similar effect produced by methionine on the growth of rats exposed to benzene and of their controls indicates that the added methionine was adequate both for growth and detoxication.

The growth-retarding effect of benzene on rats which were fed a diet containing 9 per cent of casein (42.1 gram) as well as on rats which received this diet supplemented with methionine (41.6 gram), proved that a low intake of methionine was not an important factor in reduction of growth of rats that were exposed to benzene. Therefore some other constituent or constituents of casein must be involved since the rate of growth of rats which were maintained on a diet containing 30 per cent of casein and 15 per cent of fat was not significantly reduced by exposure for twelve weeks to an atmosphere which contained 600 parts per million of benzene (2).

The incidence of leukopenia among rats which were poisoned with benzene and which received methionine was significantly less than it was among rats, also poisoned with benzene but of which the diet was not supplemented with methionine, but neither an intake of methionine nor of 30 per cent protein (2) completely prevented leukopenia among rats which were exposed to an atmosphere which contained 600 parts per million of benzene. Leukopenia was a more sensitive measure of benzene poisoning than was retardation of growth. In examination of animals of which growth has been retarded by benzene poisoning leukopenia usually is found, but leukopenia may occur in animals which are exposed to benzene without their growth being significantly retarded. However, there is a correlation between weight and leukocyte count among rats which are exposed to benzene and the diets of which are deficient in protein; this correlation is significant. The coefficient is 0.16, which is greater than 4P<sub>Er</sub> (0.11) (15).

#### SUMMARY

1. Methionine markedly increased the growth of rats which were deficient in protein, irrespective of whether the animals were exposed to benzene.
2. Methionine reduced the incidence of leukopenia in rats exposed to benzene.
3. Judged by the curve of growth and the number of leukocytes in the blood, exposure to an atmosphere which contained 600 parts per million of benzene, for

forty hours a week, was still toxic to rats which were maintained on a diet which contained 9 per cent of protein and which was enriched with methionine.

4. A decrease in the number of circulating leukocytes was a more sensitive indication of benzene poisoning in rats than was retardation of growth.

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# POST-BURN AZOTEMIA, ITS CHARACTERISTICS AND RELATIONSHIP TO THE SEVERITY OF THERMAL INJURY

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In a recent study of the blood chemistry of patients with thermal injury Walker (1, 2) observed that post-burn azotemia differed profoundly from uremic azotemia. It developed simultaneously with clinical signs of toxemia, in spite of an apparently adequate urinary excretion, and was characterized chemically by the fact that 50 to 80 per cent of the increase in the non-protein nitrogen of the plasma was due to the undetermined nitrogen fraction. All patients suffering from burns with non-protein nitrogen levels above 100 mgm. per cent succumbed. Walker suggested that development of such a type of azotemia could be used as a laboratory criterion of the degree of the toxemia and as an index of its severity. The causal relationship between the high plasma levels of undetermined nitrogen and the syndrome of toxemia was not determined.

Since the interpretation of metabolic disturbances in severely burned patients is often complicated by such variables as the nutritional history, secondary infection, and necessary therapeutic measures, it was deemed desirable to study the characteristics of post-burn azotemia in a uniform population of laboratory animals under strictly controlled experimental conditions. The data on the non-protein nitrogen partition in the plasma of burned rats which are presented in the following sections were obtained from Wistar rats of uniform weight subjected to standard scalds of known lethality (3). Clinical evidence of infection of the injured area was never observed. To test the relationship between the increase of the undetermined plasma nitrogen and the syndrome of toxemia, post-burn blood with varying degrees of azotemia was compared with normal blood as to its curative efficiency in scalded rats. The general results obtained with this procedure have been presented by McCarthy and Parkins (4).

**METHODS.** Wistar rats of  $200 \pm 10$  grams body weight were selected for the experiments. They were reared and maintained on a diet of Purina dog checkers supplemented with whole milk. The animals were scalded by means of the standardized back-burn technique described by McCarthy (3). Water was withheld for the first 12 hours and food for the first 24 hours after the burn.

Heart blood was collected from the heparinized<sup>1</sup> animals under light ether anesthesia. If the blood was used only for nitrogen analysis, it was centrifuged within 60 minutes after its collection and the plasma was drawn off. Blood utilized for both transfusion experiments and chemical assays was stored for one day in the refrigerator before a sample was removed for plasma analysis. This storage did not alter significantly the non-protein nitrogen partition in the plasma.

<sup>1</sup> One hundred Toronto units of heparin (Connaught) were injected into the femoral vein 2 minutes before cardiopuncture.

The plasma proteins were precipitated by the addition of 4.5 volumes of water, 4 volumes of N/12 sulfuric acid and one half of a volume of 10 per cent sodium tungstate per volume of plasma. The precipitate was removed by centrifugation. One milliliter samples of the supernatant fluid, corresponding to 0.1 ml. of plasma, were used for the determinations of total nitrogen (non-protein nitrogen) and urea nitrogen by means of the titrimetric ultramicro-method of Sobel, Mayer and Gottfried (5) and of amino-nitrogen by means of the colorimetric micro-method of Frame, Russell and Wilhelmi (6, 7). The difference between non-protein nitrogen and the sum of urea nitrogen plus amino nitrogen is referred to as the undetermined nitrogen.

The term as used here includes most of the uric acid nitrogen and the entire creatinine nitrogen which were determined separately in human post-burn plasma by Walker (1). Both of these nitrogen fractions constituted only a small percentage of the total non-protein nitrogen in Walker's analyses. On the other hand, the colorimetric amino acid test is not specific for  $\alpha$ -amino groups, as is the ninhydrin method used by Walker (1). The nitrogen of the secondary amino groups of di-amino acids as well as of certain amines, such as histamine and adrenalin, is therefore recorded by us as amino nitrogen.

Since in the plasma of scalded rats the increases in the amino nitrogen and undetermined nitrogen fractions show similar trends, the slight difference in the distribution of nitrogenous compounds between the two fractions does not introduce a serious objection against the comparison of the results on the post-burn plasma of man and rat. The biochemical procedure used in our study offers the advantage of permitting estimation of the non-protein nitrogen partition in as little as 0.4 ml. of plasma. This makes possible analyses upon individual rats in spite of the great hemo-concentration that results from severe burns.

**EXPERIMENTAL RESULTS.** In figure 1 the relative increases in the non-protein nitrogen fractions of the plasma of rats subjected to a 100 per cent lethal scald are compared with those following a scald of 50 per cent lethality. After both types of burn the steepest rise occurred in the undetermined nitrogen fraction which rose 4 to 5 times from its pre-burn level within the first four hours after scalding while urea nitrogen and amino nitrogen were only doubled. Up to 4 hours after the scalding no correlation was apparent between the accumulation of nitrogenous catabolites in the plasma and the severity of the burn. The blood urea nitrogen continued to increase in burns of both degrees of lethality at an identical rate. The highest urea levels were found in the lower mortality group 16 hours after scalding, i.e., at a time when all the animals exposed to the 100 per cent lethal burn had already died. In contrast, the undetermined and the amino nitrogen fractions ceased to increase 4 hours after the milder burn returning gradually to normal levels within the following 48 to 96 hours, while continuing to increase after the more severe burn up to the time of death of the animals. It follows that the increases in the latter two non-protein nitrogen fractions reflect the severity of the burn whereas the urea nitrogen levels do not.

The severity of scalds at a given temperature can be experimentally controlled by varying either the area of the burn or the time of exposure to the scalding

agent. While similar proportions of the total body mass may be damaged by either procedure, the types of tissue injured will not be identical as the time of exposure largely determines the depth to which the heat penetrates. It seemed of interest, therefore, to ascertain whether the depth of the burn would influence the nitrogen catabolism of the animals. The lethal burn utilized for this purpose was produced by scalding 30 per cent of the body surface of rats for a period of 35 seconds. This burn appeared to be more severe than the 45 per cent surface-15 second scald inasmuch as it reduced the 12 hour survival rate of the rats from 50 per cent to 13 per cent. The nitrogen analyses were done upon individual rather

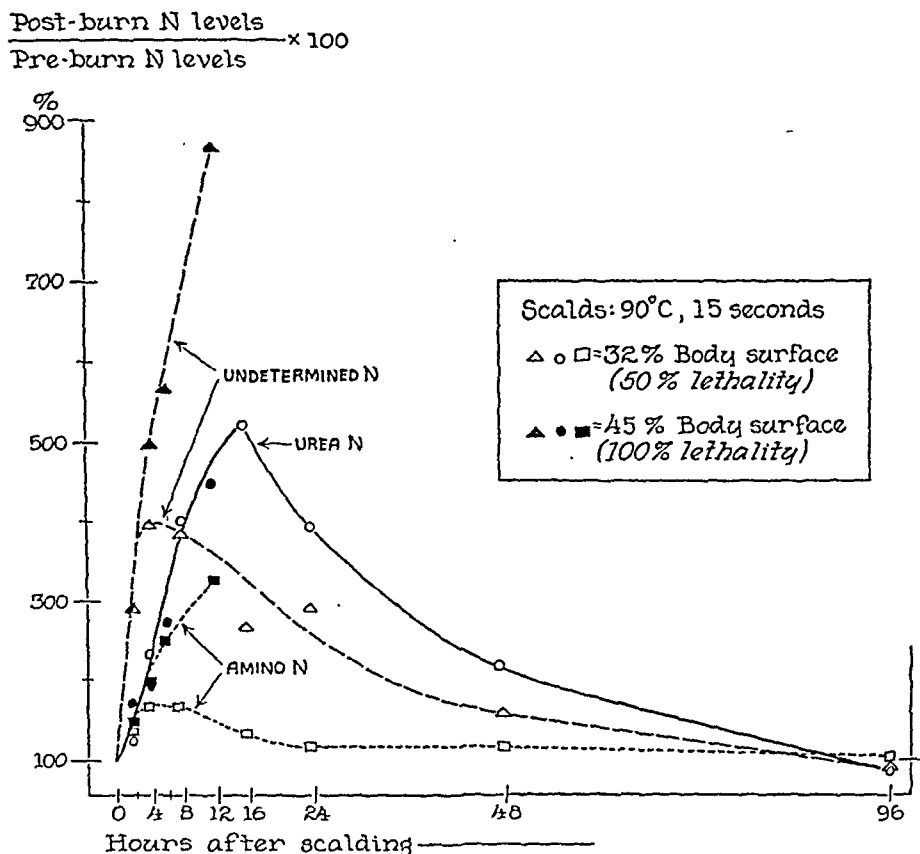


Fig. 1. The relative increases in the non-protein nitrogen fractions of plasma following scalding. Note that the urea levels for both types of scalds are represented by a single curve. The 100 per cent lethal scald (heavy dots) terminates with the 12 hour value.

than upon pooled plasma in order to get information on the range of variation of the nitrogen levels.

Figure 2 demonstrates that the urea nitrogen levels were not significantly different in the three types of burn under comparison. This is in accordance with our previous conclusion as to the absence of correlation between severity of scald and rise of urea nitrogen. Attention is called to the large scatter range of the assays upon individual rats subjected to the 30 per cent-35 second scald as contrasted to the close agreement of the analyses upon pooled plasma used in studying the effects of the first type of lethal scald. These results indicate that

mixed plasma, while satisfactory for the study of general trends of metabolism, is not a suitable material for judging the prognostic significance of biochemical alterations in the individual case.

From figure 3 it is evident that the undetermined nitrogen levels furnish a sharp distinction between the three types of burn. The longer exposure type of lethal scald (30 per cent-35 seconds) is characterized by an especially steep initial rise of this nitrogen fraction followed by a broad maximum around 8 hours. The range of variation of the individual values is small. The mode of the

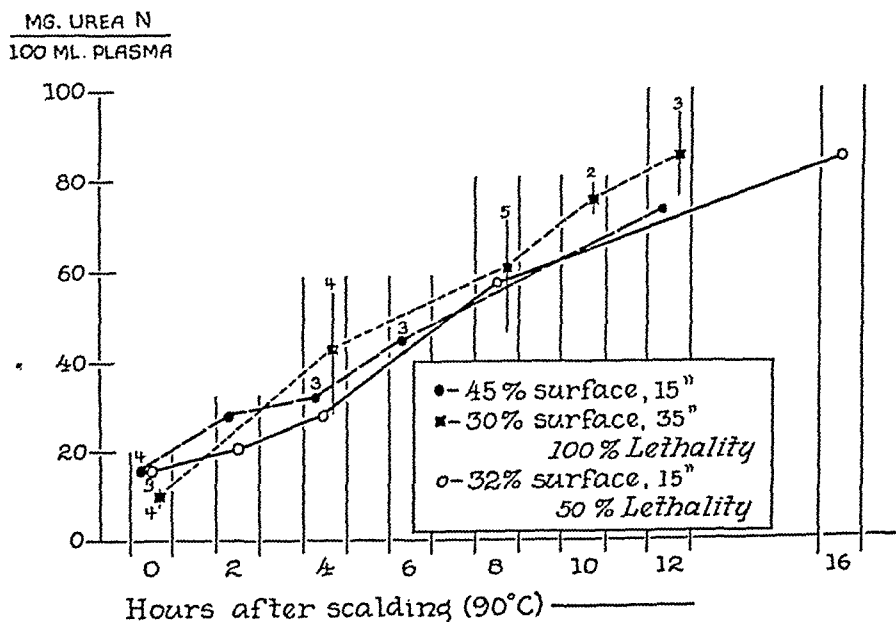


Fig. 2. Urea nitrogen levels following burns of varying severity.

The experiments were done upon plasma from the following types of blood: 1, stored pooled blood from several animals (45 per cent-15 second scald); 2, fresh pooled blood (32 per cent-15 second scald), and 3, fresh blood of individual rats (30 per cent-35 second scald).

Note that for clarity of the graphic presentation units of time are represented by the distances between the pairs of heavy vertical lines instead of by definite points. Within the vertical columns thus formed the values for each of the three types of burn are plotted at arbitrary, but constant, positions with reference to the abscissae. This arrangement prevents the overlapping of the three plots. The light vertical lines through the points of the curves indicate the scatter range of the individual determinations while the numerals refer to the number of specimens examined.

curve resembles that of the 50 per cent lethal scald. However, the greater severity of the burn is clearly revealed by the absolute height of the maximum. The slight terminal fall of the curve occurred in spite of the continuous deterioration of the condition of the animals. In fact, one rat in each the 8 and the 12 hour group and both rats sacrificed at 10 hours were moribund.

The amino nitrogen curve, which is also given in figure 3, exhibits a trend quite similar to that of the undetermined nitrogen curve. It appears, however, that the amino nitrogen levels did not differentiate between the three degrees of burn as distinctly as did the undetermined nitrogen levels. It is doubtful whether

the comparatively low maximum of the amino nitrogen level obtained with the 30 per cent-35 second burn can be considered as a characteristic of this long exposure, small surface type of scald since the amino nitrogen levels of the normal control rats<sup>2</sup> of this series were also lower than those of the controls examined simultaneously with the two other types of scald. The relative increase of about 350 per cent following the 30 per cent-35 second burn was similar to the increase of 325 per cent obtained with the 45 per cent-15 second burn (see fig. 1).

McCarthy and Parkins (4) have shown that whole blood collected 4 hours after a lethal standard burn had no curative effect when given intravenously to rats 2 hours after a 50 per cent lethal scald. In contrast, administration of normal

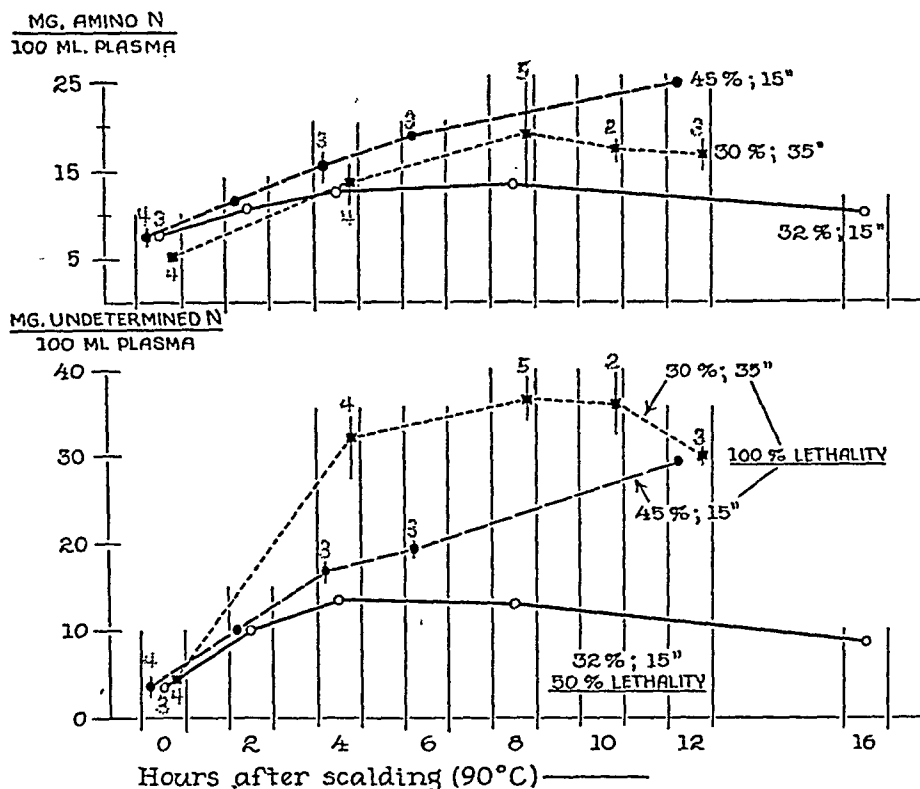


Fig. 3. Amino nitrogen and undetermined nitrogen levels following burns of varying severity. For details compare with the legend of figure 2.

whole blood markedly reduced the mortality rate of the group. The experimental evidence suggested that the difference in efficacy of the normal and the post-burn blood rested in the composition of the plasma. It was postulated that the hemodynamic action of the transfused post-burn blood counterbalanced any injurious effect on the part of the plasma to such an extent that ineffectiveness rather than increased mortality resulted from the treatment.

<sup>2</sup> The urea nitrogen levels were also significantly lower than in the previous control series. The average amino nitrogen levels for normal rats reported in two publications (6, 8) from the New Haven laboratories show differences similar to those observed by us. The cause of these variations is obscure.



In view of the close relationship between undetermined nitrogen levels and severity of burn it was thought possible that the suspected toxicity of post-burn plasma was correlated with its undetermined nitrogen content. Accordingly the therapeutic efficacy of the whole blood which had served for the plasma nitrogen analyses on the 45 per cent surface-15 second scald (figs. 1 and 3) was tested in the manner outlined by McCarthy and Parkins (4). The 2 hour post-burn blood was approximately as effective as normal blood. The 4, 6 and 12 hour specimens, which contained 70, 100 and 150 per cent more undetermined plasma nitrogen, had all lost their therapeutic efficacy. There was no evidence of increasing toxicity of post-burn blood with increasing levels of the undetermined plasma nitrogen.

COMMENT. In studying the metabolic changes in shock after burns Harkins and Long (8) reported that the plasma amino nitrogen of rats rose proportionally to the severity of the scald. The relatively greater increase of the undetermined plasma nitrogen found by us has not been previously noticed in experimental burns<sup>3</sup>. Amino nitrogen and undetermined nitrogen constitute a larger proportion of the non-protein nitrogen of cells than of plasma. Hence a rise in the plasma levels of these fractions must be expected to follow thermal tissue destruction and to parallel to a certain extent the degree of local injury. In addition, it is known (9, 10) that shock per se leads to increased amino nitrogen levels in the blood which are caused by a rise of the protein catabolism in anoxic peripheral tissues and by diminished oxidation of amino acids in the anemic liver. Such a double mechanism may also be operative in the accumulation of the undetermined nitrogen. The time course of the rise in the plasma levels of these two non-protein nitrogen fractions represents the balance between the rate of release of nitrogenous metabolites into the blood stream and their rate of elimination by enzymatic conversion into urea and by urinary excretion.

In severely burned rats urinary excretion is negligible for at least 12 hours after the injury. The changes in the non-protein nitrogen pattern are therefore governed mainly by the rates of formation and metabolization of the nitrogenous catabolites. During the first post-burn hours the inflow of amino nitrogen and, especially, of undetermined nitrogen into the circulating blood exceeds the animal's capacity of urea formation. The initial post-burn azotemia is characterized by a subnormal ratio of urea nitrogen to total non-protein nitrogen. With increasing length of time this ratio rises gradually to normal or slightly above normal levels. It finally reaches, in the survivors from milder burns, the high values characteristic of uremic azotemia. Such high ratios may persist for 24 to 48 hours following scalding.

The burn azotemia in the rat has two biochemical features in common with the toxemia syndrome of burned patients: 1, plasma non-protein nitrogen levels

<sup>3</sup> Lambret, Driessens and Warembourg (11) found increased polypeptide nitrogen levels in post-burn blood from dogs. This nitrogen fraction consists probably of higher protein split-products which are precipitated from whole blood by phosphotungstic or tungstic acid but are soluble in trichloroacetic or sulfosalicylic acid (12). Apparently such nitrogenous compounds do not contribute to the undetermined plasma nitrogen studied by us, as the nitrogen content of tungstic acid filtrates of post-burn plasma did not significantly differ from that of trichloroacetic acid filtrates.

above 100 mgm. per cent in all fatal cases; 2, a close correlation between increase of the undetermined nitrogen fraction and lethality. Contrary to the findings in man, however, this type of azotemia is limited to the shock phase of burn. No biochemical or clinical signs of toxemia develop in rats that recover from shock. Even severe scalding of rats does not result in blister formation and in oozing of plasma from the burned area. The scalded skin remains intact for more than a week after the burn. Clinically demonstrable infections do not occur. The different response to thermal injury of this species as compared to man may account for the absence of the toxemia syndrome.

#### SUMMARY

In view of recent clinical evidence for the existence of a typical post-burn azotemia which is largely due to a rise of the undetermined plasma nitrogen, the non-protein nitrogen partition was studied in the plasma of rats subjected to standard scalds of known lethality. Non-protein nitrogen, urea nitrogen and amino nitrogen were determined, the difference (non-protein nitrogen - (urea nitrogen + amino nitrogen)) being referred to as undetermined nitrogen.

To test for toxicity of nitrogenous metabolites, post-burn blood with varying degrees of azotemia was compared with normal blood as to curative efficacy in burned rats. The following results have been obtained:

1. The increases in the undetermined nitrogen and amino nitrogen of plasma reflect the severity of burn whereas the urea levels do not. The proportionally largest increase occurs in the undetermined nitrogen fraction, the levels of which provide the most sensitive biochemical criterion of the severity of burn. This finding is in accord with the results described by Walker (1, 2) in man.

2. Contrary to the experience in man, azotemia with large increases of the undetermined nitrogen is restricted to the shock phase of burn. It is followed in the recovering animals by a uremic azotemia which gradually disappears with returning urinary excretion. Possible reasons for the different responses to thermal injury have been discussed.

3. There was no evidence of an increasing toxicity of transfused post-burn blood with increasing levels of the undetermined plasma nitrogen.

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# VISUAL SENSATIONS AROUSED BY MAGNETIC FIELDS<sup>1</sup>

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Visual sensations aroused by physical stimuli other than light are called phosphenes, and their production by the application of electric currents to the eye has long been known. Electromagnetic fields may also elicit phosphenes; a relatively obscure fact which d'Arsonval (1896) appears to have been the first to record. Thompson (1910) unaware of previous work, rediscovered this effect of magnetic fields using 1,000 Gauss at 50 c.p.s. and described the sensation as a colorless flicker which was brightest in the peripheral parts of the visual field. Dunlap (1911) and Magnusson and Stevens (1912) confirmed this description and made the additional observation that the 25 cycle field was more effective than the 60. For our work we used alternating magnetic fields of variable frequency and compared the properties of the sensations thereby produced with those produced by passing sinusoidal electric currents through the head.

**METHODS.** The magnet had 397 turns of number 16 copper wire. The dimensions were: internal diameter 10.5 cm., external diameter 20.3 cm., length 7.3 cm. A laminated iron core 5.3 x 2.9 x 37 cm. was placed inside the winding. Current was supplied by a generator, and the strength was adjusted by a variable transformer. The frequency was measured with a calibrated magneto and varied from 10 to 90 c.p.s. The field strength was calculated from the readings of an A.C. voltmeter connected to a small search coil, calibrated in a field of known strength and frequency. All values are in R. M. S. Gauss. Using 20 amperes we were able to obtain up to 900 Gauss. The subject was seated with his temple close to, but not necessarily in contact with, the core of the magnet; under optimal conditions the phosphene could be seen when the temple was several centimeters from the core. The subject fixated steadily on a white spot placed in the center of a dark grey background.

To produce phosphenes by electrical stimulation current from a beat frequency oscillator was applied between an electrode on the side of the forehead and one on the back of the forearm. These electrodes were copper discs 3 cm. in diameter, covered with cloth soaked in saturated sodium chloride solution, and were held in place with adhesive tape. Small changes in inter-electrode resistance should not affect the results since current, not voltage, was measured. The currents used never exceeded 1 milliampere and we were not troubled with pain at the site of the electrode.

<sup>1</sup> A preliminary note on this work appeared in *Fed. Proc.* 5: 110, 1946. We wish to thank Dr. K. S. Lion and Prof. F. O. Schmitt, Massachusetts Institute of Technology, for their helpful interest in this work, and the Baruch Committee on Physical Medicine for providing the laboratory equipment.

The wave forms of both the magnetic fields and the electric currents were sinusoidal at all frequencies used. Most of the work was done upon ourselves but several others have seen the phosphenes.

**RESULTS.** The sensations aroused by sinusoidal magnetic and electric stimuli were qualitatively the same at the strengths used by us. At a field strength of 760 Gauss, or a current of 0.3 mA., a stimulus at 60 c.p.s. produced a definite but not very intense shimmering in the periphery of the visual field. This was usually colorless but was occasionally described as faintly tinted with blue or yellow. The phosphene was seen both with the eyes open and shut, and at these intensities lasted only a few seconds, gradually fading until it was no longer perceptible. As the intensity of the stimulus was increased the flickering appeared brighter and persisted for a longer time. It filled a larger part of the visual field, approaching the central part from the periphery, but we have never seen it occupy the point of fixation. The flicker was difficult to detect if the illumination of the field was intense.

The iron core of the magnet concentrated the field in a comparatively small area, and by using a search coil it was found that the field intensity had fallen considerably even a few centimeters away. Therefore, for convenience most experiments were carried out with the temple close to the core. No phosphenes were aroused by placing the coil over the occipital region of the skull even when high field strengths were used. A more localised field was obtained by using a core which came to a point a few millimeters in diameter, and this concentrated the most intense part of the field over a small area of the retina. The sensations thus aroused were referred to the opposite quadrant of the visual field; for example, the flickering was seen medially if the magnet was applied laterally, and above if it was applied below. These relationships suggest that it is the retina itself which is stimulated by the magnetic field rather than the optic nerve.

Firm pressure on the eyeball causes complete but temporary loss of vision in a few seconds. This procedure also renders the eye insensitive not only to electric stimuli (Finkelstein, 1894), but to magnetic stimuli as well, an observation that supports the view that the locus of excitation is within the eyeball.

If the eye is subjected to strong magnetic or electric stimulation a period follows during which excitation is more difficult. This can be shown by subjecting the eye to strong stimuli of 10 to 60 seconds' duration, and measuring the time at which the eye again responds to slightly suprathreshold stimuli, applied every 5 seconds. The threshold was taken as that stimulus strength which produced a visual sensation in half the trials. Threshold determinations were not at all easy; nevertheless as the figures in table 1 show it was evident that a period of subnormality followed the application of strong stimuli and lasted as long as 30 seconds in some instances. The period of subnormality was longer when the fatiguing stimulus was maintained for 60 seconds rather than for 10 seconds, even though the sensation had disappeared within the first ten seconds. When one eye was stimulated maximally with the magnetic field for 60 seconds no change was detected in the threshold of the opposite eye.

In some experiments the duration of the sensation produced by a steadily

maintained stimulus was measured with a stop watch. As Pollock and Mayer (1938) showed using A.C. stimulation, the duration is greater at the low frequen-

TABLE 1  
*Recovery from previous stimulation*

SUBJECT	STIMULATION	TEST STIMULUS (% THRESHOLD)	TIME FOR RECOVERY AFTER A STIMULATION	
			of 10 sec.	of 60 sec.
W	Magnetic	110	0-3	15-29
	Magnetic	110	0-3	10-15
B	Magnetic	120	3-5	15-20
K	Magnetic	120	not done	6-15
	Magnetic	124		20-25
	Electric	128		25-30
		137		5-10
		108		30-40

In the magnetic experiments a fatiguing stimulus of 790 Gauss at 60 c.p.s. was employed. In the electric, 0.67 milliampere at 60 c.p.s. was used.

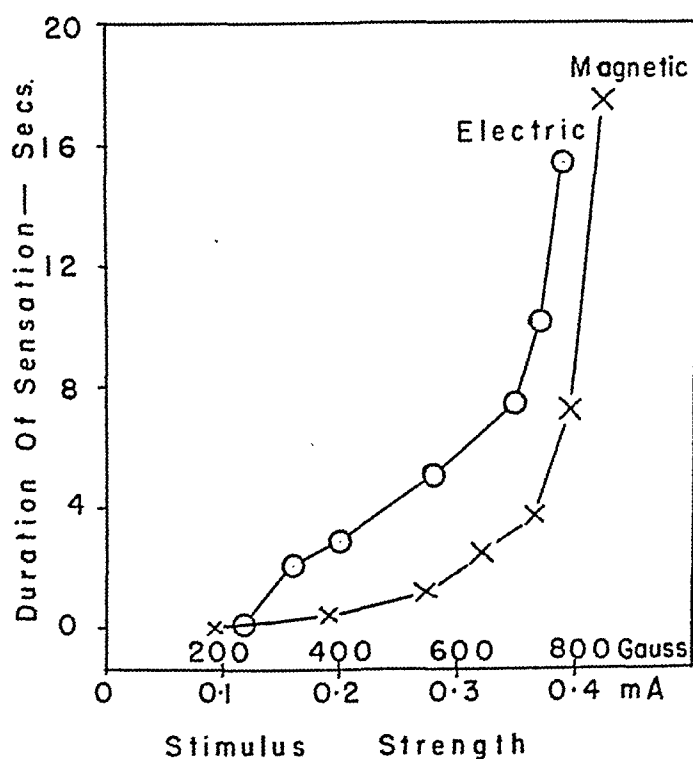


Fig. 1. Duration of the phosphene as a function of the intensity of the stimulus. Frequency 30 c.p.s. Subjects E.G.W. (magnetic) and H.B.B. (electric).

cies. This also seems to be true for magnetic stimulation. The duration also depends on the strength of the stimulation; the sensation persists longer the higher the stimulus intensity (fig. 1).

The sensation fades most readily if fixation of the eye is carefully maintained, but is prolonged if the eye wanders. Changing the point of fixation during the course of an experiment prolongs the sensation. For example, subject W. was tested with magnetic stimulation (130 per cent threshold, 60 c.p.s.). Fixation on point *A* produced a sensation lasting for 7 seconds, whilst in another trial fixation on point *B* produced a sensation lasting for 7.5 seconds. Points *A* and *B* subtended an angle of  $52^{\circ}$  at the eye. In a third trial the subject fixated on *A*, and as soon as the sensation had vanished fixated on *B*; in this case the sensation was seen for a total of 12 seconds. Similar results were obtained when the eyes were moved in the dark. Blinking also prolongs the sensation. The influence of eye movements on magnetic phosphenes was known to earlier workers (Beer, 1902).

In order to determine the effect of opening or closing the eyes, the threshold was determined in the dark after it had reached a steady value. It was found that closing the eye might raise the electric threshold by as much as 100 per cent, but did not affect the magnetic, a fact to be considered in the design of experiments.

**DISCUSSION AND SUMMARY.** The phosphenes produced by both electric and magnetic stimulation are 1, maximal in the periphery of the visual field; 2, colorless; 3, abolished by pressure on the eyeball; 4, subject to fatigue; 5, induced by frequencies up to at least 90 c.p.s.; 6, prolonged by eye movements. The phosphenes differ in only one respect, namely, that shutting the eye raises the threshold for electric stimulation but not for magnetic. In view of these similarities we believe that both forms of stimulation probably activate the same neural elements.

As to the locus of excitation we believe that this is retinal, for otherwise we cannot explain the effects of localised magnetic stimuli, pressure on the eyeball, and movements of the eyeball, all of which profoundly influence the phosphene.

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# THE EFFECT OF DARK ADAPTATION AND OF LIGHT UPON THE ELECTRIC THRESHOLD OF THE HUMAN EYE

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When an electric current of adequate strength is passed through the eye, a visual sensation or phosphene is perceived. With strong currents the sensation may be colored, but when strengths close to threshold are used a whitish flash is perceived which is strongest in the peripheral part of the visual field, and closely resembles the flashes produced by alternating magnetic fields. In our previous paper (Barlow, Kohn and Walsh, 1946) some evidence was given localising the structures sensitive to magnetic fields and alternating currents to the retina. In order to define more closely the structures sensitive to electric currents we have investigated the effect of dark adaptation and of light upon the threshold for electric stimulation.

This problem has been investigated previously by a number of workers but the results are somewhat contradictory; for instance, of the authors who stimulated with D.C. three (Muller, 1897; Nagel, 1904; Bruckner and Kirsch, 1913) found no change in threshold during dark adaptation, whilst the later workers (Bogolovsky, 1934; Bouman, 1935; Schick, 1935) found an increase. More consistent results were obtained by the workers who stimulated with condenser discharges; all five agreed that the rheobase increases during dark adaptation (Verrijp, 1925; Achelis and Merkulow, 1929; Fischer and vom Hofe, 1932; Gersuni, 1935; Katasima, 1939). A discontinuity in the strength duration curve for the light adapted eye was found by Achelis and Merkulow (1929) but not confirmed by later investigators, and Bouman suggested that this kink was the result of random variations. A study of these papers shows that the chronaxie does not change, or changes very little with dark adaptation; on the other hand there is apparently a ten fold increase in the rate of accommodation during dark adaptation (Bouman, 1939). One author (Lasareff, 1924) stimulated with faradic current and detected no changes, and Cords, (1907) using A.C., also reported no change in the maximum frequency at which flicker could still be perceived.

Three papers have dealt with the effect of increased illumination on the threshold. Fischer and vom Hofe (1932) and Schick (1935) found an increase in rheobase, whilst Bogolovsky (1934), who covered the eyes during the determinations of threshold, found a transient decrease.

A study of these papers coupled with our own results leads us to believe that most of the discrepancies are more apparent than real, being due to differences in technique. During the course of this work it has become apparent that light plays a dual rôle. Depending on the attendant circumstances, both with respect

to previous as well as immediate exposure, light may either lower or raise the electric threshold.

**METHODS.** A conventional circuit was employed to deliver condenser discharges at a rate of 20 to 30 per minute. Switching was accomplished by an automatic cam operated contact breaker and stimulation was maintained for the shortest time necessary for the determinations. A 100,000 ohm resistance was connected in parallel with the electrodes to minimise polarisation, and a series resistance, of the same value, was connected in the circuit to minimise the effects of changes in skin resistance. The electrodes were cloth covered copper discs, 3 cm. in diameter, moistened with saturated sodium chloride solution and held firmly in place on the forehead and forearm.

The subject sat erect fixating a small mark on a cream colored wall whose reflection factor at  $90^\circ$  was 0.75. The illumination was varied by using electric lamps of different strengths and was measured with a comparison photometer calibrated against a Macbeth Illuminometer. Dark adaptation was achieved either by extinguishing all lights or by placing a small opaque mask over the subject's eyes. The course of dark adaptation was followed by light stimuli from an adaptometer (Wald, 1941). Threshold was taken as the peak current eliciting a flash in half the trials.

To obtain consistent values it was necessary to control certain details of the procedure:

1. The interval between successive shocks should be at least two seconds, because at shorter intervals fatigue is encountered.
2. The position of the eyeball relative to the rest of the orbit affects the threshold. It is therefore necessary to control the position of both the eye and the head during the determinations.
3. The eyelids should be open during the determination, in the light, or in the dark, since closing them may raise the threshold 100 per cent.
4. The subject should be comfortable; experiments should be relatively short in order not to tire him.

**RESULTS.** The effect of light and of dark adaptation upon the electric threshold of the eye is illustrated by figure 1. In this instance, typical of 18 such experiments, the subject was exposed to light at zero time, and his threshold determined 14, 25 and 36 minutes thereafter ranged between 0.3 and 0.34 milliamperes (50 msec. discharges). At 44 minutes all light was extinguished, and 1 minute later the threshold had dropped to 0.12 milliamperes. Five minutes later it had risen to 0.25, and subsequently it rose smoothly, reaching a plateau value of 0.44 milliamperes after approximately 40 minutes of darkness. The electric thresholds in this experiment were not all equally easy to obtain. In the light and during the first part of dark adaptation the threshold seemed fairly sharp. After 10 to 15 minutes in the dark, however, the "threshold region" broadened, that is to say, the subject found it increasingly difficult to decide just when the phosphene was no longer elicited.

For comparison, the visual dark adaptation curve, determined in the same experiment, is also shown in figure 1, the scale of thresholds being plotted in log



millimicrolamberts. While the electric threshold rose the light threshold dropped from more than 4 to less than 2 log units in the course of about 40 minutes.

These results are not substantially affected by changes in skin resistance since similar ones were obtained using subcutaneous needle electrodes. Changes in pupillary diameter are likewise unimportant as was shown by the use of physostigmine and homatropine.

Changes in the time constant of condenser discharge, tested from 3 to 50 milliseconds, changes the absolute but not the relative values. An example of this is shown in figure 2, where the curves for 10 and 50 milliseconds are compared.

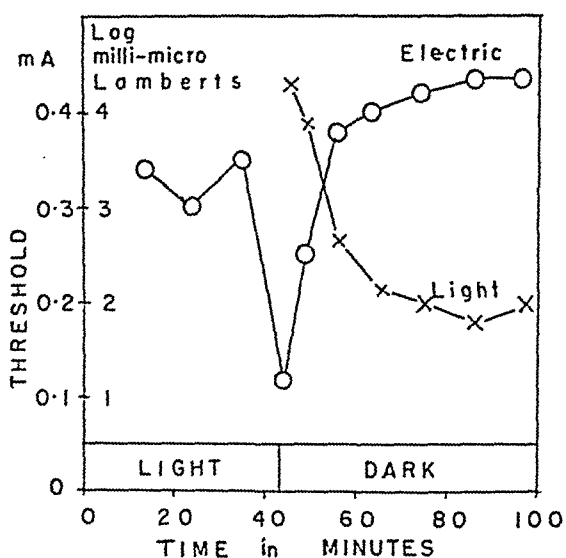


Fig. 1

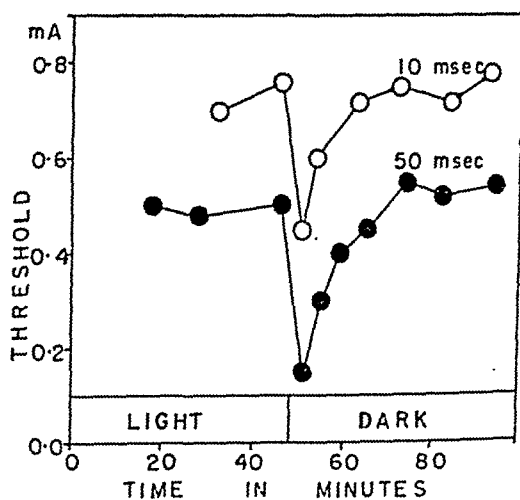


Fig. 2

Fig. 1. Changes in the electric and light thresholds of the eye during dark adaptation. The electric thresholds (50 millisecond condenser discharges) in milliamperes are plotted as the open circles. The light thresholds in log millimicrolamberts are plotted as crosses. Following the exclusion of light at the 43rd minute, there was an immediate fall in electric threshold, which then gradually rose during the next 40 minutes while the light threshold was falling.

Fig. 2. Electric thresholds for 10 and 50 millisecond condenser discharges during dark adaptation.

The longer stimulus merely results in a lower absolute threshold throughout the course of the curve. The threshold determined as a function of duration of condenser discharge showed that the chronaxie was approximately 1.8 msec. both at full dark adaptation and at adaptation to one foot-candle. In other experiments direct current pulses lasting about 0.8 second were used as stimuli and similar results were obtained. In this instance, however, a flash was seen both when the circuit was made and also when it was broken. For the dark adapted eye the make and break thresholds were equal, or nearly so, but for the light adapted eye the break threshold was considerably higher than the make. When the dark adapted eye is exposed to light there is an immediate large in-

crease in the threshold. Following this the threshold falls in the course of a few minutes, as the dazzling effect of the light diminishes.

The rapid initial decrease in electric threshold on extinguishing the light was studied further. In one experiment illustrated in figure 3 this change was determined at two levels of light intensity. The thresholds determined in the light are plotted as open circles, those determined during brief interpolated periods of darkness as black circles. During the first 7.5 minutes the adapting intensity was 5 foot candles and thereafter 355 foot candles. It is apparent that the fall in threshold on switching off the light was greater at the higher intensity

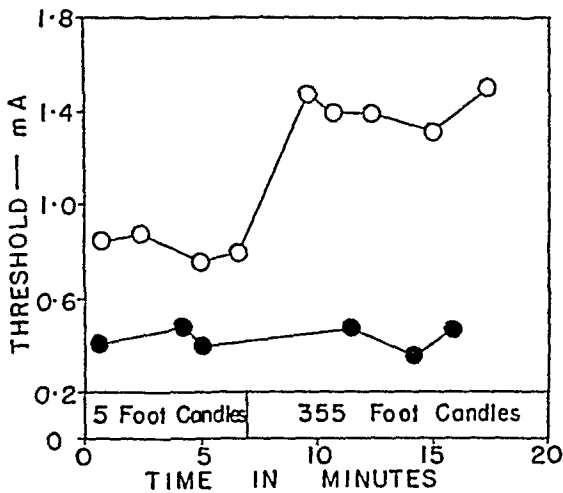


Fig. 3

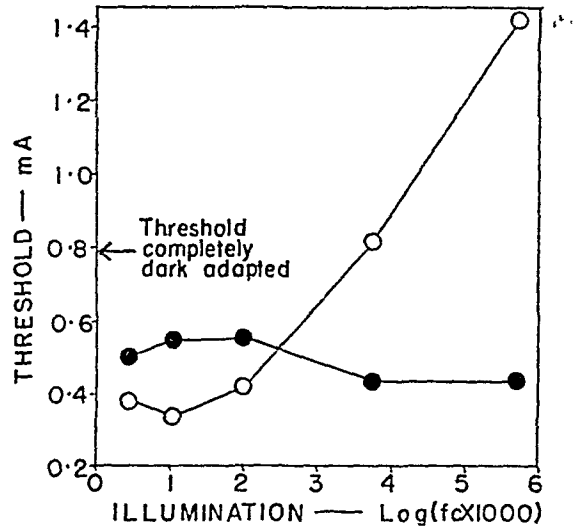


Fig. 4

Fig. 3. Thresholds in the light (open circles) and immediately after turning out the light (black circles) at two different light intensities. The threshold in the light rises when the adapting light intensity is increased, but the threshold taken in the dark remains relatively constant.

Fig. 4. The electric threshold of the eye (in milliamperes) as a function of adapting light intensity ( $\log [\text{foot candles} \times 1000]$ ). After light adaptation, the electric threshold was determined in the light (open circles) and also just after extinguishing the light (solid circles). The threshold of the completely dark adapted eye is also indicated.

of illumination. The thresholds during the brief intervals of darkness were surprisingly constant.

In another series of experiments the electric threshold (i.e., the minimum peak current required to elicit a flash) was determined with the observer exposed to a number of different light intensities (fig. 4.) The thresholds obtained while looking at the illuminated field (open circles) rose as the intensity of the illumination was increased. On the other hand thresholds determined immediately after covering the eyes fell slightly as the illumination increased (black circles), but even at the lowest illuminations they were considerably lower than the threshold for the completely dark adapted eye. One further point may be observed; whilst at moderate and high intensities of illumination withdrawal of light leads

to an immediate fall of electric threshold, at the lowest intensities withdrawal of light leads to an immediate rise.

DISCUSSION. The electrical threshold rises as the illumination to which the eye is exposed is increased (fig. 4). This observation has an obvious parallel in the fact that the threshold for seeing a light superimposed upon a bright background also rises as the illumination of the background is increased (Weber effect).

When the eye is plunged into darkness the electrical threshold for arousing phosphenes immediately falls; it then slowly rises over the course of 20 to 40 minutes, as dark adaptation occurs, to reach a plateau. The biphasic nature of this change in threshold may explain some of the discrepancies hitherto reported.

Whilst the precise locus of excitation remains unknown the slow rise in threshold during dark adaptation would suggest that the photochemical system of the retina is involved. This possibility was also suggested by the work of Kitasima (1939), who found an elevation of rheobase in a subject placed on a vitamin A deficient diet. It is interesting to observe that intensities of light as low as ca 0.004 foot candle (so low that they could only stimulate the rods) reduce the electrical thresholds of the eye (fig. 4).

#### SUMMARY

1. The threshold of the human eye to electric currents produced by condenser discharges has been measured.
2. The threshold of the light adapted eye is increased by increasing the illumination.
3. When illumination is suddenly withdrawn from the eye adapted to more than about 0.1 foot-candle, the electric threshold falls rapidly then rises slowly as the visual threshold falls.
4. For adaptation in the range 0.004 to 0.1 foot-candle, withdrawal of light causes a small abrupt rise in electric threshold, followed by a further slow rise to the full dark adaptation level.
5. Changes in pupillary size, skin resistance and chronaxie are not responsible for these results.

We wish to thank Dr. K. Lion and Prof. F. O. Schmitt, Massachusetts Institute of Technology, for their continued interest in this work. We are indebted to Dr. Geoffrey Dawes for reading our manuscripts, to Dr. G. Wald for the loan of apparatus and for several useful suggestions, and to the Baruch Committee for their support.

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# FAILURE OF "ENTEROGASTRONE" TO PREVENT RUMENAL ULCERS IN THE SHAY RAT

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The need for a simple, reliable, short-term bio-assay method for the anti-ulcer factors in intestinal and urinary extracts is pre-eminent. At the present time the only means available for the demonstration of the anti-ulcer properties of these substances is the Mann-Williamson (M-W) dog (1, 2). This method requires at least six months of observation before it can be decided whether or not the material being assayed is effective in preventing ulcers. Attempts to prevent other kinds of experimentally induced peptic lesions, namely, the histamine in beeswax ulcer (3) and the chick gizzard erosion (4), with intestinal extracts known to be effective in the M-W dog have not been successful.

Recently (1946), Friedman and Sandweiss (5) reported a method for bio-assay of urogastrone based on the gastric secretory depressant activity of that preparation when injected intravenously into 24-hour fasted rats. Subsequently, Pauls, Wick and MacKay (6), utilizing their modification of the technique for rapid production of "ulcers" of the rumen of starved rats by ligation of the pylorus, as reported by Shay and co-workers (7), claimed reduction in extent or prevention of such ulceration following the intravenous administration of "a sufficient dose" of urogastrone. These investigators suggested that their procedure might be adapted for use as a rapid assay method for any hormonal anti-ulcer agent. Consequently, the study herein reported was undertaken in an attempt to develop some simple, short-term bio-assay of "enterogastrone", based on its anti-ulcer property.

**METHODS.** The technique used in this study was essentially that proposed by Shay, with the modifications added by Pauls and co-workers. Sprague-Dawley albino male rats ranging in weight from 120 to 180 grams were used. Where long term treatment was carried out calculations were made from growth curves in order to have the animals in this weight range at the end of the treatment and starvation period. The rats, kept on a stock laboratory diet, were given daily intramuscular injections of an aqueous solution of injectable "enterogastrone" preparation (8) with doses ranging from 25 to 500 mgm./kgm. (table 1). Various concentrations of the preparation were used to keep the volume small and about the same for all animals. Controls received daily intramuscular injections of physiologic salt solution in approximately the same volume as the enterogastrone solution injected. Treatment periods ranged from one day (injection at time of operation) to thirty days. Forty-eight hours prior to operation the rats were isolated in individual cages with wide-mesh screen bottoms and permitted only water *ad lib*. At the end of the 48-hour starvation period the abdomen was

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opened under ether anesthesia and the pylorus ligated with a coarse, braided-silk ligature, care being taken to avoid the blood vessels. The abdomen was closed and the wound painted with collodion to prevent buffering of the gastric juice by blood and serum licked from the wound. Eight to ten hours later the animals were sacrificed, the stomach contents aspirated with a needle and syringe, noting the volume of gastric contents. The stomach was then excised, opened along the greater curvature and secured to a cork-board with pins. After washing the mucosal surface with saline solution the specimen was studied under a dissecting microscope, a count being made of the number of ulcers present without regard to size.

**RESULTS.** The results in 47 rats treated 1, 3, 5, 7, 9, 11, 20 or 30 days with doses of 25 to 500 mgm. of "enterogastrone" per kgm. indicated that rumenal ulcers occurred in all except one animal. The average number of ulcers per rat ranged from one in an animal treated with a single 400 mgm./kgm. dose to 29 in a rat treated with one 500 mgm./kgm. dose. In general there was no correlation between the size of the dose or the length of treatment and the number of ulcers that developed. The average volume of gastric contents at the time the animal was sacrificed, though following no mathematical progression, was lowest (6.5 cc.) in the rat treated with the highest dose of the preparation and highest (12.1 cc.) in the control group.

To determine the effectiveness of administration of "enterogastrone" by other parenteral routes, two groups of rats (12 animals) were treated at the time of operation by administration of "enterogastrone" intraperitoneally or intravenously (tables 2 and 3). All the intraperitoneally treated animals developed ulcers. Three of the five intravenously treated rats developed ulcers; one died following a 500 mgm./kgm. injection and the other did not develop ulcers, having received 400 mgm./kgm. However, the effect of such a large intravenous dose in this latter animal produced such a toxic reaction that it would be erroneous to deduce that this is the assay level. In fact, the marked toxicity, as manifested by cyanosis and prostration, which occurred in all the intravenously treated animals makes it difficult to evaluate the lower volume of gastric contents (1 to 7 cc.) in this group. Either generalized toxicity or the gastric secretory depressant property of the preparation, or both, may be responsible. It should be noted that the dosages used here are much larger, on the basis of milligrams per kilogram, than are ever used in the assay of these preparations in dogs.

However, even if it should prove possible to prevent the rumenal ulcer of rats by the gastric secretory depressing action of intestinal extracts, the question of the relationship between the anti-ulcer factor and the gastric secretory depressing factor demands consideration.

Although present evidence indicates that urogastrone (9) and enterogastrone (10) protect against ulcer formation by some mechanism other than depression of acid secretion, the close relationship between gastric secretory depressing factors and ulcer preventing factors is supported by much evidence. In the first place, only those tissues and fluids which contain an acid secretory depressant have been shown to contain an anti-ulcer principle. All extracts, urinary

TABLE 1

*Shay rats administered intramuscular "Enterogastrone"*

NO. OF RATS	AV. WEIGHT	DOSE	NO. OF DAYS TREATED	AV. VOL. GASTRIC CONTENTS*	NO. DEVELOPING ULCERS	AV. NO. ULCERS PER RAT	REMARKS
	grams	mgm/kgm		cc.			
8	125	25	1, 3, 5, 7, 9, 11, 20, 30	10.2	8	15	
8	130	50	1, 1, 3, 5, 7, 9, 11, 30	10.3	8	21	7 & 11 day treated rats perforated
8	123.5	75	1, 3, 5, 7, 9, 11, 20, 30	9.9	7	18	3 day treated rat did not develop ulcers
9	128.7	100	1, 1, 3, 5, 7, 9, 11, 20, 30	9.2	9	15.8	7 day treated rat perforated
3	159	200	1, 20, 30	10.3	3	20	
1	166	400	1	10.0	1	1	
1	180	500	1	6.5	1	29	
9	134	Controls 0.35 cc. phys. saline sol.	1, 1, 3, 5, 7, 9, 11, 20, 30	12.1	9	11.2	

\* At time sacrificed.

TABLE 2

*Shay rats administered intraperitoneal "Enterogastrone"*

NO. OF RATS	AV. WEIGHT	DOSE	NO. OF DAYS TREATED	AV. VOL. GASTRIC CONTENTS*	NO. DEVELOPING ULCERS	AV. NO. ULCERS PER RAT	REMARKS
	grams	mgm/kgm		cc.			
1	170	50	1	10.0	1	33	
1	166	100	1	13.0	1	30	
1	166	200	1	12.0	1	22	
1	166	400	1	11.0	1	1	Small pin-point size ulcer
1	176	500	1	8.0	1	15	
1	165	Control 0.4 cc. saline	1		1	Innumerable	Mucosa in shreds. Perforated

\* At time sacrificed.

and intestinal, which have been efficacious in preventing ulcer contain a gastric secretory depressant even though its action may not be manifest in the dose used or by the route administered (parenteral urogastrone and "enterogastrone" concentrates prevent ulcers when administered in doses too small to depress gastric secretion, and intestinal extracts containing "enterogastrone" are effective orally (11) in preventing ulcer although they do not exert any gastric secretory

TABLE 3

*Shay rats administered intravenous "Enterogastrone"*

NO. OF RATS	AV. WEIGHT	DOSE	NUMBER OF DAYS TREATED	AV. VOL. GASTRIC CONTENTS*	NO. DEVELOPING ULCERS	AV. NO. ULCERS PER RAT	REMARKS
	<i>grams</i>	<i>mgm/kgm</i>		<i>cc.</i>			
1	150	50	1	7	1	26	
1	156	100	1	5	1	14	
1	156	200	1	1	1	13	
1	156	400	1	2	0	0	No ulcer developed
1	206	500	1				Lethal dose
1	156	Control 0.4 cc. saline	1	8.4	1	24	

\* At time sacrificed.

TABLE 4

*Number of ulcers on basis of days treated*

NO. OF ANIMALS	DAYS TREATED	NO. OF ULCERS
9	1	13.5
4	3	8.0
4	5	14.2
4	7	18.2
4	9	19.0
3	11	21.3
4	20	11.5
5	30	22.0

depressing action even when administered in huge doses by the oral route). No preparation free of gastric secretory depressant has been demonstrated to be capable of preventing ulcer. Furthermore, Sandweiss has shown that the anti-ulcer effectiveness of urine extracts apparently decreases concomitantly with decrease in gastric secretory depressant potency (12). These facts indicate that if the gastric secretory depressant and anti-ulcer factors are separate substances then their occurrence and chemical behavior are remarkably similar.



Until the relationship between the anti-ulcer factor and the gastric secretory depressing factor is clarified conclusions regarding the anti-ulcer potency of an extract should not be based solely upon its potency as a secretory depressant.

#### SUMMARY AND CONCLUSION

"Enterogastrone" administered parenterally in relatively large doses for varying periods up to 30 days does not afford protection against rumenal ulcers which form in rats following pyloric ligation. Neither is it possible to correlate the number of ulcers produced with the length of treatment or the dose of the preparation administered.

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# THE CREATININE, INULIN AND HIPPURATE CLEARANCE IN THE RAT<sup>1</sup>

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Although the creatinine clearance has been used to measure the glomerular filtration rate of man (1) and dog, (2), it apparently has not been employed in the study of the rat's renal function. Furthermore only two studies (3, 4) have been published on the inulin clearance of the rat and none have been published about the hippurate clearance. It seemed important then not only to determine the creatinine, inulin and hippurate clearances in the rat but also to determine the relationship of these clearances to urine flow and to study the significance of the creatinine clearance in the rat. The results of the succeeding study make it clear that all of these clearances vary directly with the urine flow and also that the creatinine clearance is a measure of glomerular filtration in the rat.

**METHODS.** Fifty-one large male albino rats fed Purina Dog Chow with supplementary feeding of green vegetables were utilized for this study. Seventy creatinine, 65 inulin and 45 hippurate clearances were done. The creatinine and inulin clearances were concurrently determined in 38 rats; the creatinine and hippurate clearances in 24 rats and the creatinine, inulin and hippurate clearances in 9 rats.

For the determination of the creatinine clearance, each rat at 9:00 a.m. was given 10 cc. of H<sub>2</sub>O containing 20 mgm. of creatinine by stomach tube, his tail was cut and a thin rubber tourniquet placed over the tail proximal to the incision. The rat was placed in a urine collecting cage until 10:00 a.m. at which time urine was expressed from his bladder; he was then anesthetized, the tail tourniquet removed and 1.0 to 1.75 cc. of blood was obtained. The rat then received 5 cc. of H<sub>2</sub>O containing 10 mgm. of creatinine, placed back into the cage and the urine collection begun. At 11:00 a.m. the animal was given 5 cc. of H<sub>2</sub>O containing the same amount of creatinine given at 10:00 a.m. without removing him from his cage. At 12:00 or exactly two hours after urine collection had begun, the rat was taken from his cage, anesthetized and a second blood sample obtained. This procedure also was used for the determination of the inulin and hippurate clearance in the rat too except that at 9:00 a.m. inulin (500 mgm.) or hippurate (100 mgm.) was given subcutaneously and only 10 cc. of H<sub>2</sub>O given by mouth. The advantages of this procedure were 1, that it extended over a long enough period of time to ensure probable renal hemodynamic balance; 2, that a large enough output of urine to offset any significant error from possible loss of several drops was obtained; 3, that the animals were in no pain and were unanesthetized during the actual collection (preliminary experiments indicated

<sup>1</sup> Aided by a grant from the Dazian Foundation for Medical Research.

that the clearances were not influenced by the very brief duration of anesthesia used to collect the first blood sample), and 4, clearance studies could be made with blood concentrations determined both at the beginning and end of the collection period. If a combined creatinine and inulin clearance were done, the same procedure was carried out except that inulin (500 mgm.) was injected subcutaneously at 9:00 a.m.

Creatinine in blood and urine was determined by the method of Folin and Wu (5). Inulin in plasma and urine was determined by the method of Hubbard and Loomis (6) except a 60 per cent HCl solution was used to hydrolyze the inulin in any sample. Plasma and urine hippurates were analyzed according to the method of Smith et al. (7). All clearance values have been expressed in cubic centimeters per 100 grams of body weight per hour.

**RESULTS.** A. *The significance of the creatinine clearance in the rat.* Our studies indicated that the creatinine clearance of the rat was a measure of the latter's glomerular filtration rate. This was suggested first by the fact that the inulin and creatinine clearances were approximately the same. Thus (see table 1) at a urine flow ranging from 0.1 to 0.5 cc. per hour per 100 grams body weight, the average creatinine clearance of 12 determinations was 18.5 cc. per hour per 100 grams of body weight (range: 12.0 to 29.2 cc.) and the average inulin clearance in 20 determinations at the same range of urine flow was 19.4 cc. (range: 6.7 to 28.2 cc.). The inulin-creatinine clearances were done together in six rats. At a range of urine flow of 0.5 to 1.0 cc. per hour, the average creatinine clearance was 28.3 cc. (range: 17.9 to 41.8 cc.) and the average inulin clearance, 28.9 cc. (range: 21.1 to 39.0 cc.). The inulin-creatinine clearances were done together in 19 rats. Likewise the similarity of clearances was maintained in a range of urine flow from 1.0 to 1.5 cc. per hour at which rate the average creatinine clearance was 39.1 cc. (range: 23.0 to 66.0 cc.) and the average inulin clearance, 36.8 cc. (range: 29.0 to 47.0 cc.). In eleven rats, the clearances were done together. Even when a urine flow of from 1.5 to 2.0 cc. per hour was obtained the average creatinine clearance (41.8 cc.) was approximately the same as that of the inulin clearance (41.1 cc.).

The creatinine clearance in the rat also was found to be independent of the plasma level of creatinine,—at least in the range studied (1.0 mgm. per cent to 26.6 mgm. per cent). No higher blood concentration of creatinine could be obtained in the rat without incurring severe diarrhea.

B. *The relationship of creatinine, inulin and hippurate clearances to urine flow.* Early in this study it became apparent that not only the creatinine but also the inulin and hippurate clearances varied directly with the urine flow. Inspection of table 1 demonstrates this well for whereas the average creatinine and inulin clearances were approximately 18 cc. per hour at urine flows ranging from 0.1 to 0.5 cc. per hour, they progressively increased with increasing urine flow until their average clearances were approximately 41 cc. at urine flows varying from 1.5 to 2.0 cc. per hour. As a matter of fact, the rather wide range of clearance values at any range of urine flow was not so much due to variability in the clearance rates as it was to the individual variations of urine flow in single

experiments within the urine flow ranges used for tabulation of results. In other words, at a given urine flow, the clearance of either inulin or creatinine was relatively stable and reproducible.

TABLE 1  
*Creatinine, inulin and hippurate clearances at increasing urine flows*

U.F.	0.1 cc. to 0.5 cc.				0.5 cc. to 1.0 cc.				1.0 cc. to 1.5 cc.				1.5 cc. to 2.0 cc.			
	Rat	CC	IC	HC	Rat	CC	IC	HC	Rat	CC	IC	HC	Rat	CC	IC	HC
23	18.5	18.0			01	32.2	31.0		23	42.2	42.0		18	36.2	32.0	180
27	16.4	15.0			23	20.0	23.0		96	45.5	46.0		30	41.0	51.3	220
24	19.5	19.0			00	24.7	27.0		28	26.0	36.0		94	42.3	42.0	230
43	14.2	8.2			00	21.2	27.0	145	45	23.0	32.0		97	36.8	28.0	222
02	16.2	18.7			00	25.0	21.1	71	22	58.5	36.0		29	44.0	57.2	175
29	19.2	16.6			04	27.0	29.0		22		33.0		75	46.8	41.0	200
14		25.0			42	19.0	21.0		00	32.2	35.0		39	39.0	38.9	230
67		15.7			42	35.5	33.0		74	53.5	47.0		96		30.3	
67		6.7			11	26.6	27.0		42	44.5	46.0	118	28		36.9	
73		13.1			24	26.3	28.0		19	39.6	36.0		58		53.0	
73		17.8			24	22.8	28.0		20	35.4	38.0		59	51.3		195
73		25.8			67	34.5	35.0		27	38.8	39.0		59			200
09		25.0			03	29.6	29.0		13		29.0		93	37.8		240
50		25.8			14	41.5	39.0		24		30.0	111				
31		14.3			73	23.9	30.0		24		31.5					
45		28.2			17	38.6	29.0		25		32.4					
03		19.7			74	34.6	30.0		95	66.0						
25		27.6			21		30.0		55	26.0						
33		22.5			02		33.8	108	09	24.9		176				
33		25.9			84	17.9			50	32.7		188				
40	12.0		68		29	20.6			50			164				
37	17.2		60		31	23.0		124	33	26.8		109				
98	14.3		78		12	20.9		97	52	40.8		212				
48	20.6		112		72	23.2		133	52	43.0		205				
52	24.8		117		72	30.4		180	43	33.4		122				
54	29.2		149		43	34.8		107	43			160				
00			77		97	39.6		214	54	37.6		184				
					39	28.2		89	72	39.4		139				
					50	41.8		145	75	39.5		202				
					50			147	31			96				
					09			123	29			150				
Average...	18.5	19.4	94			28.3	28.9	129		39.1	36.8	155		41.8	41.1	209

UF urine flow in cc./100 grams weight/hour.

CC creatinine clearance in cc./100 grams weight/hour.

IC inulin clearance in cc./100 grams weight/hour.

HC hippurate clearance in cc./100 grams weight/hour.

The plasma hippurate clearance (see table 1) likewise was found to vary directly with the urine flow. Thus the average hippurate clearance of eight determinations was 94.6 cc. per hour (range 60.0 to 149.0 cc.) at a urine flow of

0.1 to 0.5 cc. per hour; 129.4 cc. (range 70.5 to 214.0 cc.) at a urine flow of 0.5 cc. to 1.0 cc.; 155.3 cc. (range: 99.5 to 205.0 cc.) at urine flow of 1.0 to 1.5 cc.; and 209.2 cc. (range: 175.0 to 240.0 cc.) at urine flow of 1.5 to 2.0 cc. As can be seen, considerable variations in hippurate clearances at any urine flow were found but no correlation could be found between the clearance and the average plasma concentrations of hippurate employed, which ranged from 1.32 mgm. per cent to 4.75 mgm. per cent.

**DISCUSSION.** The above studies made it seem quite likely that the creatinine clearance was a measure of glomerular filtration in the rat. Its approximate equality to the inulin clearance and its apparent independence of the blood concentration of creatinine allowed of no other conclusion.

The finding of a well defined relationship between the creatinine, inulin and hippurate clearances and the rate of urine flow in the rat is at variance with the findings of Dicker and Heller (4) who stated that both inulin and diodrast clearances were independent of diuresis in the rat. It is quite possible that the rapidity with which they began their clearance determinations (30 min. after ingestion of  $H_2O$ ) together with the brevity of urine collection (10 to 25 min.) made it impossible to estimate the actual function of the kidney under conditions of equilibrium. Moreover their assumption that the average blood concentration of either inulin or diodrast could be calculated from a single terminal blood sample is to be questioned for it seems quite doubtful for example that a rapidly excreted substance as diodrast would remain unchanged for a period of 10-25 minutes approximately 60 minutes after its subcutaneous injection. As a matter of fact there is good reason to suspect that diodrast probably decreases as rapidly in rat's blood as hippurate was found to do in our experiments since it is as rapidly excreted as hippurate. Finally, the necessarily small amounts of urine collected in the majority of their experiments allowed the possibility of incomplete collections.

Although there appeared to be no question that all clearances varied with the urine flow, it is possible that the variations in urine flow were an expression of the variations in renal plasma flow as assumedly expressed by the hippurate clearance. In other words, perhaps it would be more correct to assume that the creatinine and inulin clearances as well as the urine flow varied directly with the hippurate clearance. Moreover, the rather great variation of this latter clearance would suggest that there is also considerable variation in the renal blood flow of the rat following the ingestion of large amounts of fluid.

#### SUMMARY

The creatinine clearance was studied and compared with the inulin clearance in rats. It was found that the former was probably a measure of glomerular filtration. The hippurate clearance was also determined in the rat. All three types of clearances were found to vary directly with the degree of urine flow.

The author wishes to express his thanks to Barbara Trousdale, William Cano, Frances Greenberg and Ruth Kornblum who assisted in these experiments.

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# THE RESPIRATORY RESPONSES TO ANOXEMIA OF THE NORMAL UNANESTHETIZED DOG AND THEIR CAUSES<sup>1</sup>

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No systematic study has previously been reported of the changes in the respiration of the normal unanesthetized dog during and after inspiration of gas mixtures varying progressively in oxygen content from 20.93 per cent to the lowest limit of tolerance. Such a complete study became necessary to provide a basis for comparison with the respiratory response to anoxemia under certain experimental conditions which we planned to investigate and also to reveal possible causal factors in the response other than those commonly accepted. The following experiments were done with these views in mind.

**METHODS.** Normal 10–15 kgm. dogs were trained to lie supine quietly while breathing through a mask and a set of valves already described (1). Except during brief intervals of struggling which occurred with oxygen concentrations below 6 per cent, the animals were subjected to no physical restraint other than slight support of the mask and loose cloth ties about the legs. The dogs were kept under uniform conditions of diet and activity and used in the post-absorptive state. Most of the experiments were performed in an air-conditioned room at 20° to 25°C; experiments performed at higher room temperatures were discarded when panting or hyperpnea of any type was present.

The gas mixtures to be inspired were made by dilution of room air with nitrogen, in one or more 90 liter Tissot spirometers. Analysis in the Haldane-Henderson apparatus (2) of more than 100 mixtures so made indicated that an oxygen concentration within 0.3 per cent of the desired value, over the range of 18 per cent to 3 per cent, could be counted upon. By using two spirometers, alternately filling one while the animal breathed from the other, the experiments could be prolonged for any period desired.

The expired air was collected in a 120 liter Tissot spirometer and the movements of the bell were recorded on a slow kymograph. Expiratory volume, tidal air and rate were obtained by analysis of the resulting records in minute intervals. This method of collection and recording makes it possible to record an unlimited volume with infrequent short gaps in the record while emptying the spirometer or changing the kymograph paper, usually a matter of less than a minute.

Except as otherwise noted the routine of an experiment was as follows: The dog, breathing air, rested supine upon a padded animal table during a five to

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ten minute rest period. This was followed by a six minute collection of the expired air and a recording of the spirometer movements. Immediately thereafter the spirometer valves were adjusted so that the animal breathed a low oxygen mixture and the collection of the expired air and the recording of the spirometer movements were made for twenty minutes. At the end of this period the spirometer valves were adjusted so that room air again was inspired and the expired air collection with recordings was continued without interruption, for a ten minute recovery period. Pulse rates were taken, by palpation of the femoral artery, at frequent intervals throughout the experiment.

**RESULTS.** In table 1 the changes in the minute volume, the rate of the respiration and in the tidal air, during the anoxic period, are summarized for two dogs (A and G). These were selected from five dogs which were studied because they typify the variations exhibited by all the different dogs. In some, as in dog A, the rate of the respiration played a lesser part in effecting the anoxic hyperpnea than in others, i.e., dog G. The figures express the per cent of change from the control. This method of expression makes the results more comparable since it minimizes the inevitable individual and daily variations.

The control minute volumes average typically  $2.6 \pm 0.2$  liters (dog A). The averages (table 1) include six experiments at each concentration of oxygen, except those at 4 per cent in which only two experiments are averaged.

The experiments are divided into two groups for the purpose of description of the characteristics of the respiration during the anoxic period. One group includes all concentrations down to and including 8 per cent of oxygen, since the behavior of the respiration is qualitatively similar in this group. The second group comprises concentrations of oxygen of 6 per cent and below, since here the respiratory behavior differs from that in the first group. The characteristics of the respiration will be described separately for each period of the experiment.

*Concentrations of Inspired Oxygen of from 16 to 8 per cent Inclusive.* *The period of anoxia.* Figures 1 and 2 illustrate, in typical experiments, the changes in the respiration and the pulse rate during and after inhalation of 10 and of 8 per cent of oxygen respectively. Results on all animals at six levels of oxygen from 16 per cent to 8 per cent differ only quantitatively from this picture.

In every case (table 1), at inspired oxygen concentrations of 16 to 8 per cent inclusive, the minute volume rises to a peak within the first 2 to 3 minutes after the start of breathing the low oxygen mixture, following which it falls to a slightly fluctuating plateau level which is maintained above the control value throughout the period of anoxemia. The height attained by the minute volume during the peak and the plateau levels increases progressively with the decrease in the percentage of oxygen inspired. In dog G, as an example, the average increase above the control was 24.8 per cent at the peak and 15.6 per cent at the plateau with 16 per cent of oxygen, whereas it was 137 per cent at the peak and 122 per cent at the plateau with 8 per cent of oxygen. The degree of fall of the minute volume from the peak becomes less as the inspired concentration of oxygen decreases.

The rate of the respiration plays little or no part in the initial rise of the minute



TABLE 1

*Changes, from control, in characteristics of respiration at different intervals during anoxia, with different concentrations of inspired oxygen*

AVERAGE PERCENTAGE DEVIATION ( $\pm$ STANDARD DEVIATION) FROM AVERAGE OF CONTROL*								
O <sub>2</sub> insp.	Time of anoxia	Dog A			Dog G			
		Minute volume	Tidal air	Rate	Minute volume	Tidal air	Rate	
16	Peak	22.1 $\pm$ 4.8	41.5 $\pm$ 13.5	- 3.8 $\pm$ 6.0	24.8 $\pm$ 14.6	23.2 $\pm$ 13.8	9.7 $\pm$ 9.7	
	6'-9'	6.1 $\pm$ 3.5	21.7 $\pm$ 7.1	-11.3 $\pm$ 6.0	15.9 $\pm$ 11.2	11.4 $\pm$ 10.5	4.7 $\pm$ 3.7	
	10'-13'	9.2 $\pm$ 8.5	29.0 $\pm$ 15.6	-14.4 $\pm$ 10.5	19.7 $\pm$ 12.4	10.8 $\pm$ 8.0	7.5 $\pm$ 4.9	
	14'-17'	6.0 $\pm$ 3.7	19.9 $\pm$ 9.8	-9.7 $\pm$ 7.7	12.5 $\pm$ 10.4	7.9 $\pm$ 6.5	5.0 $\pm$ 7.7	
	18'-20'	4.4 $\pm$ 6.2	19.6 $\pm$ 15.3	-9.7 $\pm$ 10.0	14.4 $\pm$ 9.8	7.2 $\pm$ 5.7	6.1 $\pm$ 4.1	
14	Peak	36.9 $\pm$ 9.4	47.9 $\pm$ 14.9	3.7 $\pm$ 15.4	29.5 $\pm$ 5.2	24.5 $\pm$ 6.0	10.5 $\pm$ 7.6	
	6'-9'	19.7 $\pm$ 14.6	21.8 $\pm$ 10.5	-1.1 $\pm$ 10.2	18.8 $\pm$ 6.6	7.2 $\pm$ 7.1	11.5 $\pm$ 4.8	
	10'-13'	12.7 $\pm$ 7.1	13.7 $\pm$ 6.8	-0.2 $\pm$ 8.2	16.5 $\pm$ 11.9	5.2 $\pm$ 8.2	11.2 $\pm$ 4.5	
	14'-17'	4.4 $\pm$ 10.1	8.3 $\pm$ 13.7	-3.0 $\pm$ 13.7	14.6 $\pm$ 7.9	8.7 $\pm$ 9.3	9.5 $\pm$ 8.6	
	18'-20'	4.6 $\pm$ 9.4	10.3 $\pm$ 15.6	-3.8 $\pm$ 10.6	16.4 $\pm$ 10.7	6.4 $\pm$ 4.7	9.5 $\pm$ 7.0	
12	Peak	61.9 $\pm$ 18.2	51.9 $\pm$ 15.0	11.0 $\pm$ 9.4	46.6 $\pm$ 6.7	31.0 $\pm$ 9.2	21.8 $\pm$ 10.1	
	6'-9'	34.0 $\pm$ 11.9	16.1 $\pm$ 7.7	16.5 $\pm$ 12.7	34.2 $\pm$ 14.0	15.9 $\pm$ 12.3	16.4 $\pm$ 9.1	
	10'-13'	30.1 $\pm$ 11.2	16.9 $\pm$ 12.8	13.6 $\pm$ 16.4	33.2 $\pm$ 8.8	13.9 $\pm$ 5.0	17.7 $\pm$ 10.4	
	14'-17'	31.1 $\pm$ 10.8	12.4 $\pm$ 9.5	16.5 $\pm$ 12.4	32.3 $\pm$ 6.8	11.7 $\pm$ 7.7	19.6 $\pm$ 11.7	
	18'-20'	28.9 $\pm$ 12.6	12.6 $\pm$ 7.4	15.6 $\pm$ 14.3	33.0 $\pm$ 15.0	10.6 $\pm$ 4.6	20.9 $\pm$ 5.6	
10	Peak	94.1 $\pm$ 18.3	80.7 $\pm$ 16.8	14.9 $\pm$ 11.3	71.0 $\pm$ 16.3	44.3 $\pm$ 9.6	29.8 $\pm$ 15.5	
	6'-9'	68.2 $\pm$ 20.4	29.3 $\pm$ 19.1	32.8 $\pm$ 18.5	59.8 $\pm$ 24.3	28.5 $\pm$ 11.4	31.3 $\pm$ 13.4	
	10'-13'	69.2 $\pm$ 22.6	27.3 $\pm$ 9.6	34.1 $\pm$ 19.1	54.8 $\pm$ 18.6	20.5 $\pm$ 7.3	31.2 $\pm$ 20.2	
	14'-17'	67.1 $\pm$ 30.0	20.2 $\pm$ 7.2	38.9 $\pm$ 22.2	54.5 $\pm$ 19.2	15.6 $\pm$ 9.7	36.5 $\pm$ 17.0	
	18'-20'	68.2 $\pm$ 30.7	17.3 $\pm$ 12.5	39.9 $\pm$ 24.3	51.4 $\pm$ 16.5	17.2 $\pm$ 9.8	31.2 $\pm$ 8.8	
8	Peak	154 $\pm$ 34.1	107 $\pm$ 18.4	37.2 $\pm$ 19.9	137 $\pm$ 14.9	44.0 $\pm$ 44.6	70.5 $\pm$ 19.6	
	6'-9'	118 $\pm$ 34.7	31.6 $\pm$ 7.4	66.9 $\pm$ 31.8	118 $\pm$ 13.0	25.5 $\pm$ 13.2	77.1 $\pm$ 27.2	
	10'-13'	118 $\pm$ 42.0	30.3 $\pm$ 5.0	67.8 $\pm$ 30.5	124 $\pm$ 14.6	12.5 $\pm$ 13.8	103 $\pm$ 36.3	
	14'-17'	144 $\pm$ 42.4	24.7 $\pm$ 12.9	71.8 $\pm$ 39.0	130 $\pm$ 24.9	6.8 $\pm$ 10.5	105 $\pm$ 49.3	
	18'-20'	110 $\pm$ 44.7	22.1 $\pm$ 9.4	73.2 $\pm$ 40.4	115 $\pm$ 17.3	8.7 $\pm$ 12.5	104 $\pm$ 22.7	
6	Peak	240 $\pm$ 16.5	174 $\pm$ 11.0	35.3 $\pm$ 15.4	279 $\pm$ 62.4	72.9 $\pm$ 24.4	152 $\pm$ 33.8	
	6'-9'	209 $\pm$ 72.1	59.9 $\pm$ 11.3	96.7 $\pm$ 58.4	298 $\pm$ 101	14.7 $\pm$ 9.7	238 $\pm$ 64.6	
	10'-13'	196 $\pm$ 76.5	51.2 $\pm$ 10.4	101 $\pm$ 59.6	345 $\pm$ 141	10.9 $\pm$ 15.4	301 $\pm$ 86.9	
	14'-17'	156 $\pm$ 15.2	64.8 $\pm$ 7.7	58.2 $\pm$ 12.4	331 $\pm$ 144	11.7 $\pm$ 10.3	290 $\pm$ 113	
	18'-20'	152 $\pm$ 22.0	61.3 $\pm$ 10.7	57.1 $\pm$ 15.0	324 $\pm$ 121	13.3 $\pm$ 10.8	281 $\pm$ 89.2	
4	Peak	419 $\pm$ 77.1	322 $\pm$ 85.6	63.5 $\pm$ 70.0	597 $\pm$ 264	126 $\pm$ 34.6	350 $\pm$ 58.0	
	6'-9'	388 $\pm$ 9.2	252 $\pm$ 95.5	44.8 $\pm$ 38.2	686 $\pm$ 138	66.1 $\pm$ 44.1	368 $\pm$ 25.5	
	10'-13'	412 $\pm$ 8.5	217 $\pm$ 107	71.9 $\pm$ 61.0	643 $\pm$ 182	53.8 $\pm$ 60.2	399 $\pm$ 79.2	
	14'-17'	471 $\pm$ 4.9	207 $\pm$ 103	97.3 $\pm$ 64.7	685 $\pm$ 242	60.1 $\pm$ 48.2	390 $\pm$ 6.2	
	18'-20'	527 $\pm$ 4.3	220 $\pm$ 82.0	103 $\pm$ 53.6				

\* All figures are positive unless otherwise noted. Values for peak minute volume and tidal air represent the highest in the first five minutes; peak rate is the rate during the peak minute volume.

volume with oxygen concentrations of 16 and 14 per cent, but becomes more of a factor as the severity of the anoxemia increases. In the case of dog G, the rate increase at the peak, with oxygen concentrations below 14 per cent, is relatively greater than in dog A.

Increase of the tidal air accounts for virtually all of the initial rise in minute volume with 16 and 14 per cent oxygen and continues to be the dominant factor as the degree of anoxemia increases, except in the case of 8 per cent of oxygen on dog G, where an increased rate predominated.

The fall of the minute volume from the peak to the plateau level is usually due to a decrease in the tidal air. In no instance, however, does the tidal air fall

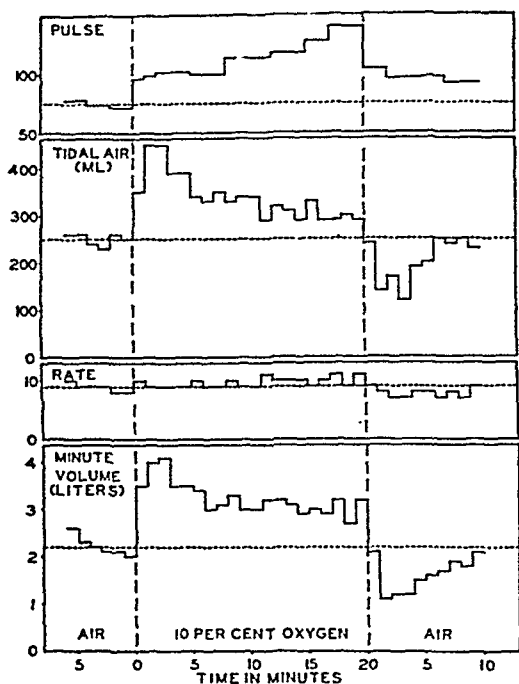


Fig. 1

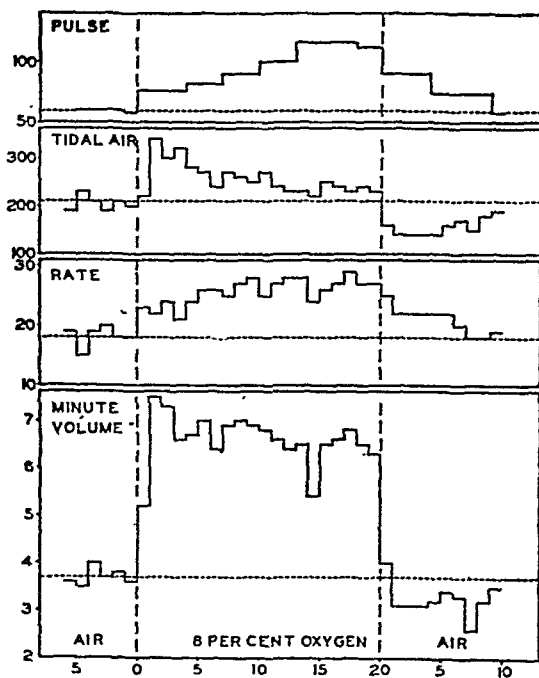


Fig. 2

Fig. 1. Changes in respiration and pulse of dog A before, during and after 10 per cent oxygen.

Fig. 2. Changes in respiration and pulse of dog K before, during and after 8 per cent oxygen.

below the control level. At concentrations of inspired oxygen which immediately produce a significant increase in the rate of the respiration, the increase is maintained or intensified (8 per cent, table 1) at the plateau level of the respiratory minute volume.

All of the dogs reacted quite uniformly in this range of inspired oxygen concentrations.

*Concentrations of Inspired Oxygen of from 6 to 4 per cent Inclusive. The period of anoxia.* When the dog inspires 6, 5 or 4 per cent of oxygen (table 1) the extent of the initial rise in the minute volume of the respiration continues to increase progressively with the decrease in the inspired oxygen tension. At 4 per cent of

inspired oxygen, increases in the minute volume of 527 (dog A) to 685 per cent (dog G) above the control have been observed within a twenty minute period. An increase in the volume of the tidal air is still an important factor in this increase. However, the rate of the respiration, in all of the dogs, participates more and more in the initial increase of the minute volume. The degree to which the rate increases and the oxygen concentration at which it becomes manifest varies in different dogs. In dog A the increase in rate is less than in dog G (table 1).

The minute volume of the respiration during a 20 minute period of breathing of 6 per cent of oxygen differs from that at higher concentrations in two respects. Following the initial rise a fall may not occur. Also, following the initial peak, secondary peaks in the minute volume may appear. The minute volume of the respiration is characterized by three general patterns: (1) it may rise quickly to a peak that is higher and maintained somewhat longer than at the higher concentrations of inspired oxygen, then fall gradually for the remainder of the 20 minute period. The fall from the peak is slighter than at the higher concentrations of oxygen (dog A, table 1); (2) it may rise steadily, with no well defined peak, for the entire 20 minute period of anoxia (dog G, table 1), or, it may rise steadily for a briefer period and maintain the high level throughout the remainder of the period of anoxia; (3) it may rise to a peak, decline, and, after a time, rise again to a peak which is usually higher than the first but occasionally lower. The number of secondary peaks depends upon the length of the anoxic period.

More prolonged experiments were performed with 6 per cent of oxygen to test whether the behavior described above was temporary. The minute volume of the respiration of dog G continued to rise steadily for an hour. The minute volume of dog A declined from the peak for 20 minutes, as described above. It then leveled off to a plateau that was maintained for another 20 minutes, then rose again to a second peak, lower than the first, followed by a gradual decline for the remainder of the hour. In another experiment on dog A the second rise did not occur until about the 80th minute. The dogs were conscious, lay quietly and showed no signs of failure in the respiration or circulation during these experiments.

When the inspired oxygen is reduced below 6 per cent, the absence of an immediate marked descent from the initial rise in the minute volume or a continuance of the rise becomes the rule and secondary rises appear more frequently and relatively earlier in the period of anoxia. The variations in the pattern of the respiration in the different dogs at 6 per cent of inspired oxygen were not so evident at 5 and 4 per cent.

Figures 3 and 4 illustrate the behavior at 4 per cent of inspired oxygen in dogs G and A, respectively.

Ten experiments were performed in which 4 or 5 per cent of oxygen was administered to the dogs for periods of time varying from 40 to 155 minutes.

The initial increase in the minute volume at concentrations of 4 and 5 per cent of inspired oxygen is greater, and the time which it rises steeply is longer, than at

the higher concentrations of oxygen. The rise is accomplished chiefly by an increased tidal air. The increase in the rate of the respiration is, however, greater than at the higher concentrations of oxygen. Following the initial rise, although the minute volume may be maintained or continue to rise for a considerable time, the tidal air decreases to a greater or lesser degree and the rate increases.

Up to impending respiratory failure, which may occur at 4 per cent of inspired oxygen, the minute volume of the respiration, following its initial rise, is greater than that at higher concentrations. The tidal air remains well above its control level throughout the anoxia.

At varying periods of time, following the initial rise of the minute volume, additional peaks appear. The time interval between the first two peaks ranged from 12 to 55 minutes with 5 per cent of oxygen and from 5 to 28 minutes with 4 per cent. During the course of the anoxia, as many as seven additional peaks

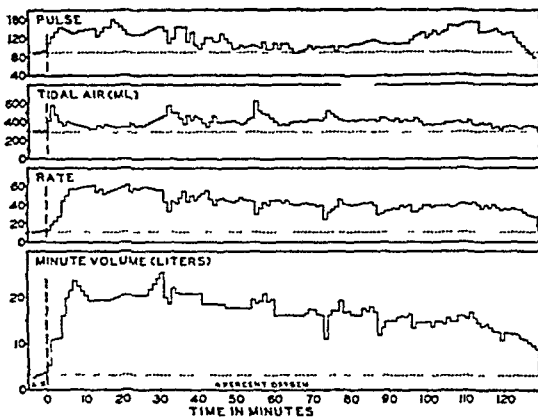


Fig. 3

Fig. 3. Changes in respiration and pulse of dog G before and during 4 per cent oxygen. Respiratory failure at 130 minutes.

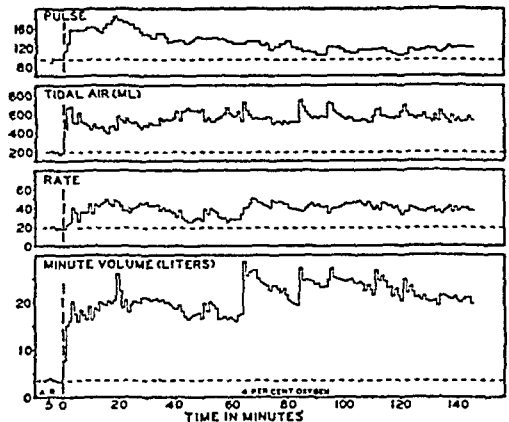


Fig. 4

Fig. 4. Changes in respiration and pulse of dog A before and during 4 per cent oxygen. No respiratory failure.

may be observed with 4 per cent oxygen, and five with 5 per cent. These occur at intervals of 3 to about 30 minutes; the majority are from 5 to 20 minutes apart with both 5 and 4 per cent of oxygen. Frequently, the maximum minute volume is not reached until the fourth or fifth peak in the case of 4 per cent of oxygen. In an experiment on dog A, with 4 per cent of oxygen, the largest volume of expired air was 34.8 liters, which was recorded during the fourth peak, twenty-eight minutes after the start of the anoxia.

Increased volume of the tidal air, with or without increased rate, accounted for the majority of the peaks in minute volume although many were produced by an increased rate accompanied by a decreased or constant tidal air.

The first evidences in these dogs of immediate and deleterious effects of anoxia appeared at oxygen concentrations of 5 and 4 per cent. About five minutes after the start of the anoxia the dogs struggled violently for a minute or two, then lapsed into unconsciousness which was maintained throughout the anoxic period

with 4 per cent. The dogs were incontinent. With 5 per cent of oxygen, periods of consciousness and struggling occurred at frequent intervals, but there were no signs of impending respiratory or circulatory failure during the time of our longest experiment at this oxygen concentration, namely, one and one-half hours. This time could have undoubtedly been extended considerably as is indicated by our experiences with 4 per cent oxygen.

Seven experiments on four dogs with 4 per cent of oxygen were continued to the point of respiratory failure, which occurred in from 40 to 130 minutes; one experiment was continued for 155 minutes without respiratory failure. Respiratory failure with 4 per cent of oxygen was preceded by a gradual lowering of the general level of the minute volume, punctuated in many instances by sudden descents to an alarmingly low level and rapid recovery. The minute volume was still 50 to 100 per cent above the control level up to a minute or two before the final respiratory failure, which was characterized by an equal diminution in both depth and rate, rather than by a gasping or a rapid shallow breathing.

The rate of the heart also diminished markedly from the height which it had attained previous to the onset of the diminution of the respiration, but it was still at its control rate or slightly below at the time of respiratory failure. Resuscitation was unsuccessful in only one instance and in this case the heart had also ceased to beat. Following the resuscitation, the dogs remained in a state of coma for periods up to two hours. In only one instance was there evidence of any prolonged after effects. About three days following an exposure to 4 per cent of oxygen for 101 minutes, the dog exhibited a rigidity of the hind legs which disappeared spontaneously in less than a week. No signs of a mental deterioration were evident in these dogs although they were under observation for many months.

A single experiment with 3 per cent of oxygen resulted, after 16 minutes, in violent struggling, vomiting and threatening respiratory failure. This concentration appeared to be the lower limit of low oxygen tolerance for the dog.

*Recovery Period in Air. Concentrations of oxygen of 16 to 6 per cent inclusive.* When the dogs are again permitted to breathe air, following a period of anoxia, the respiratory minute volume promptly falls toward or below the control value, reaches a minimum in 2 to 3 minutes and then rises to or above the control level within 5 to 10 minutes. The average per cent reduction in the minute volume from the control during the minute when it reaches its minimum value, and the variations in the tidal air and the rate of respiration are presented in table 2.

The greatest drop recorded in the minute volume was 51.4 per cent below the control figure (dog A); in most experiments it was not more than 30 per cent. Nothing resembling even a transient apnea was ever observed. The degree of the depression of the minute volume is least at the highest (16 per cent) and the lowest (6 per cent) oxygen concentrations in dogs A and G, while in dog K it does not vary significantly between 14 and 6 per cent. This behavior is illustrated graphically in figure 5, where the plotted figures of the minute volume are the average of the first five minutes of the recovery period, expressed as per cent

below the control, rather than the minimum figures given in table 2. Qualitatively similar curves are obtained if the latter figures are plotted. It is apparent that, down to concentrations of inspired oxygen of about 8 per cent, the average depression of the respiration upon breathing air increases with the intensity of the previous state of anoxia and, therefore, with the degree of anoxic hyperpnea. However, at 6 per cent of inspired oxygen, the degree of the respiratory depression in the after period is lessened. Indeed, in dog G, after 6 per cent of inspired

TABLE 2

*Changes, from control, in characteristics of respiration during minute of minimum expiratory volume of after-period*

% O <sub>2</sub> INSP.	AVERAGE PERCENTAGE DEVIATION ( $\pm$ STANDARD DEVIATION) FROM CONTROL AVERAGE, OF EXPIRED VOLUME DURING MINUTE OF MINIMUM VALUE OF AFTER-PERIOD		
Dog A	Minute volume	Tidal air	Rate
16	-15.3 $\pm$ 5.3	+4.7 $\pm$ 9.2	-25.2 $\pm$ 12.2
14	-29.8 $\pm$ 14.5	-24.2 $\pm$ 12.4	-17.1 $\pm$ 11.4
12	-23.3 $\pm$ 12.4	-21.2 $\pm$ 14.9	-19.2 $\pm$ 13.7
10	-29.6 $\pm$ 14.0	-32.4 $\pm$ 11.4	-13.5 $\pm$ 8.3
8	-30.2 $\pm$ 13.7	-44.6 $\pm$ 6.2	+1.9 $\pm$ 16.2
6	-22.9 $\pm$ 7.4	-31.8 $\pm$ 4.3	-14.3 $\pm$ 5.9
Dog G			
16	-13.1 $\pm$ 6.0	-13.7 $\pm$ 7.3	-12.1 $\pm$ 11.4
14	-16.1 $\pm$ 7.5	-12.0 $\pm$ 7.2	-11.0 $\pm$ 9.7
12	-17.9 $\pm$ 5.9	-20.1 $\pm$ 6.7	-10.9 $\pm$ 14.8
10	-28.8 $\pm$ 3.7	-25.8 $\pm$ 7.1	-17.0 $\pm$ 5.6
8	-28.9 $\pm$ 7.3	-35.2 $\pm$ 2.4	-10.8 $\pm$ 15.4
6	-13.9 $\pm$ 7.7	-50.4 $\pm$ 3.7	+9.3 $\pm$ 11.9
Dog K			
16	-16.2 $\pm$ 7.9	-15.0 $\pm$ 4.6	-13.5 $\pm$ 12.2
14	-21.6 $\pm$ 9.0	-15.1 $\pm$ 9.5	-19.6 $\pm$ 18.9
12	-24.3 $\pm$ 13.9	-28.8 $\pm$ 7.7	-9.4 $\pm$ 17.3
10	-28.3 $\pm$ 7.6	-27.4 $\pm$ 2.3	-15.9 $\pm$ 11.1
8	-26.4 $\pm$ 7.4	-37.0 $\pm$ 6.6	-5.0 $\pm$ 5.9
6	-27.1 $\pm$ 8.4	-30.6 $\pm$ 5.4	-13.9 $\pm$ 9.9

oxygen, the average of the minute volume of the respiration, during the first five minutes, does not fall significantly below the control value.

In the after-period, the volume of the tidal air during the minute of minimal volume of respired air (table 2), decreases below the control progressively with the decrease in the inspired oxygen concentration; the greatest reduction was 56 per cent (dog G). In dogs A and K the depression of the tidal air tends to be less at 6 than at 8 per cent.

The average rate of the respiration during the minute of greatest respiratory depression is slightly and variably lower than the control rate. The greatest reduction found was 43 per cent (dog A); in most experiments it was about one

fourth of this value. At the lowest concentrations of oxygen the rate may slightly exceed the control (dog A, 8 per cent and dog G, 6 per cent).

The depression of the minute volume upon breathing air following an anoxia is due both to a decreased depth and rate of the respiration, but, usually, more to a decrease in the tidal air than in the rate. The rate may be still above (dog A, 8 per cent and dog G, 6 per cent) or deviate minimally (dog K, 8 per cent) from the control level, at the point of maximum depression of the respiration, when the volume of the tidal air is least. It follows that, in the after-period, the rate falls more slowly than the depth of the respiration (fig. 2). The pulse rate shows a similar lag in its fall to the normal when breathing of air is resumed (fig. 2).

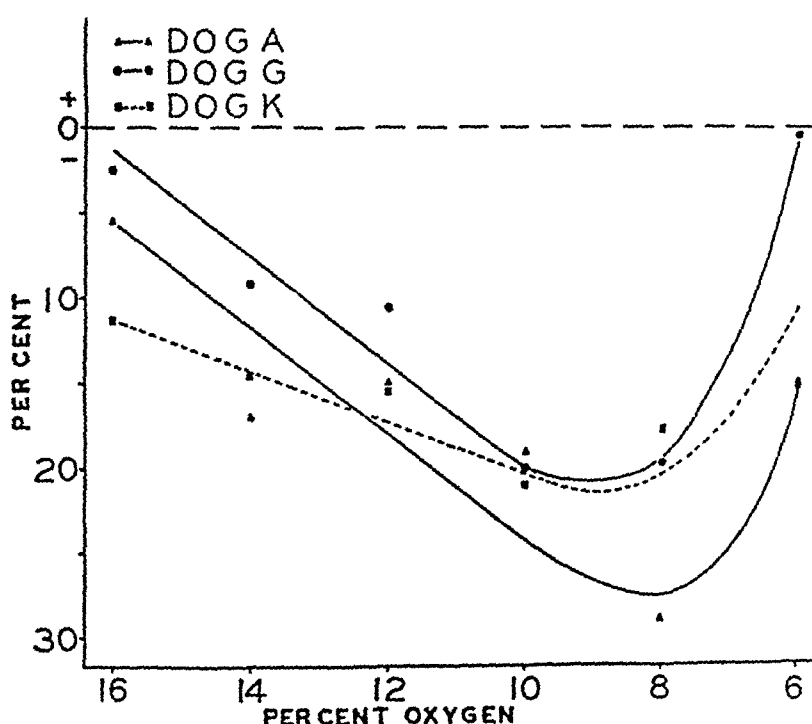


Fig. 5. Abscissae = concentration of inspired oxygen. Ordinates = average per cent deviation from control average of average expired volume during first 5 minutes of after-period.

No records of the respiration after inspiring concentrations of oxygen below 6 per cent were made. However, at no time following inspiration of 5 per cent of oxygen was there anything remotely resembling an apnea. The same is true of one experiment of 155 minutes' duration in which 4 per cent of oxygen was inspired and which was terminated short of respiratory failure.

**DISCUSSION.** The respiration during an anoxemia is the resultant of a balance of factors, some tending to increase, others to diminish the minute volume, each varying in degree with the severity of the anoxia.

Those increasing the respiration are the chemoreceptor reflex stimulation and the acid products of metabolism, accumulating to an abnormal degree within the cells of the centers as a result of the anoxia.

The physiological factors which may diminish the respiratory minute volume and which are inevitable consequences of a reflex hyperpnea are a low tension of carbon dioxide and a low hydrogen ion concentration of the blood, which will tend to reduce but not necessarily cancel the acidity within the central respiratory cells. Also, there is present to an increasing degree, with progressive lowering of the oxygen tension of the blood, a pathological depressant action upon all of the cells of the brain. The presence of the latter effect is first evidenced by unconsciousness of the dog, at concentrations of inspired oxygen of 5 and 4 per cent, and ultimately by the stoppage of the respiration and the heart.

The immediate and marked increase in the minute volume of the respiration, within the first three minutes of an anoxemia, is due mainly or entirely to a reflex stimulation of the respiratory centers, originating in the chemoreceptors of the carotid artery and the aortic arch; denervation of these receptors prevents this type of response (3, 4). This does not exclude the probability that the reflex stimulation, even during this early period, may be reinforced by central chemical stimulation, especially when very low tensions of oxygen are inspired.

The influences which are operating to modify the respiration, following the immediate chemoreceptor response, at the various concentrations of inspired oxygen, produce a different pattern above and below 6 per cent.

The descent from a well defined initial peak of the respiratory minute volume, which is characteristic of inspired oxygen percentages of 16 to 8 inclusive, is most probably due mainly to the loss of carbon dioxide and the consequent decreased hydrogen ion concentration of the blood, reflected in similar directional changes in the cells of the centers. This is essentially the explanation first advanced by Haldane and Poulton (5). As a result of the hypocapnic depression of the respiratory centers, the chemoreceptor reflex stimulation cannot maintain the minute volume at the peak level. The plateau level represents a new balance between the factors stimulating and those depressing the respiration. If carbon dioxide is added to the inspired low oxygen mixture at this time the minute volume rises.

Unpublished data on the oxygen saturation of the arterial blood indicate that the stimulus to the chemoreceptors at the plateau level at inspired oxygen percentages above 6 per cent, is as great or greater than earlier in the period of anoxia.

No evidence exists that the chemoreceptors are depressed by a low oxygen tension. On the contrary, evidence in this paper shows that they are increasingly stimulated. Depression must, therefore, reside in the central mechanisms of respiration, which consequently would be less responsive to direct and reflex influences.

In contrast to the behavior above 6 per cent, at 6 per cent of inspired oxygen and lower, it is quite evident that following the immediate chemoreceptor reflex stimulus, other stimuli to the respiration appear sufficiently powerful to overcome all of the factors which are depressing the respiration. Indeed, this additional stimulation may be exerted early enough in the period of anoxia to prevent the appearance of a definite initial peak and depression, or to make them very transient.



The progressively increased minute volume of the respiration with the lowering of the inspired tension of oxygen is most probably the result of an increased stimulus to the chemoreceptors by the progressively lower tension of oxygen in the arterial blood. On the other hand, the maintenance of the initial increase in the minute volume, at inspired oxygen concentrations of 6 per cent and below, in contrast to the descent to a lower plateau level at higher concentrations, and in many cases the slow continued rise throughout the period of anoxia, must be due to stimuli superimposed upon the chemoreceptor stimulation. A continually increasing reduction in the oxygen tension of the arterial blood which is necessary for the stimulation of the chemoreceptors, in the face of a maintained or increasingly effective alveolar ventilation and a constant tension of inspired oxygen, is hardly possible. Hence, the site of the increased stimulus to the respiration must be sought elsewhere than in the carotid and aortic chemoreceptors. On the basis of our present knowledge there remains only the respiratory centers in the medulla and pons. This greater and more sustained increase in the minute volume of the respiration occurs in spite of an undoubtedly greater opposition due to a lesser tension of carbon dioxide and great alkalinity of the blood and a greater depression of the central respiratory cells by the lowered tension of oxygen. The stimulus to the respiration must overcome these depressing effects and the more so as the tension of oxygen in the inspired air is lowered.

Further evidence of a stimulation in addition to the chemoreceptor reflex is afforded when, following the anoxic period, the dog is again permitted to breathe atmospheric air. If the chemoreceptor reflex were alone responsible for the anoxic hyperpnea, on the basis of prevailing concepts, its removal by breathing air should result in a diminution of the respiratory volume which should bear a direct relationship to the degree of the pre-existing hyperpnea and resulting hypocapnia and should finally cause an apnea. On the contrary in these unanesthetized dogs the respiratory depression, in the after-period, is least following the highest and lowest tensions of inspired oxygen, although the anoxic hyperpnea increases progressively as the inspired tension of oxygen falls. When the dog breathes room air, following the inspiration of 6 per cent of oxygen, the minute volume is often but slightly or insignificantly depressed below the control level, and in either case, after the first drop, may rise above the control figures. Apnea has never occurred in any of our experiments. The greatest recorded reduction of the minute volume was about half of the control value. This means that in spite of the removal of the chemoreceptor stimulation, and, in spite of the depression of the respiration due to a residuum of low carbon dioxide tension and increased alkalinity, and, most probably, a persisting degree of central anoxic depression, a powerful stimulus to the respiration persists. This stimulus is greater the greater the degree of the pre-existing anoxia; it is apparent that under the prevailing conditions it cannot be carbon dioxide. This behavior of the dogs, in the after-period, agrees with that obtained upon unanesthetized man by Horvath, Dill and Corwin (6).

The repeated peaks of the minute volume of the respiration, which appear

earlier and more frequently as the inspired oxygen is decreased below 6 per cent, are a definite evidence that the ventilation is dependent upon a fluctuating balance of stimulating and depressing factors. These peaks may or may not exceed the height attained by the minute volume within the first minutes of the anoxia; they may rise from a fluctuating level of ventilation or from one that is constantly rising. In any case, a central stimulus in addition to that from the chemoreceptors appears to be the most satisfactory explanation of their occurrence. The fall from the peak would be explained by an oxidative removal of the stimulating products. The stimulus could hardly be due to carbon dioxide, as this must be diminishing with a rising alveolar ventilation, while the chemoreceptor stimulus would be decreasing with an increase in the oxygen tension of the arterial blood. The latter would also lessen the central depression due to the anoxia.

The nature of the respiratory stimulus which superimposes itself upon the reflex mechanism of the chemoreceptors has been the subject of investigation and will be reported later. Its persistence and relatively slow disappearance following relief of the anoxemia is a strong indication that it is chemical in nature.

The dominant factor in the attainment and the maintenance of the increased minute volume, and in the rise to the peaks, during an anoxemia in the dog, is an increased volume of the tidal air. Increase in the rate of the respiration becomes significant only at the lowest tensions of inspired oxygen; it also tends to increase as the anoxic period progresses. However, the depth of the respiration never decreases below the control level. A rapid shallow respiration is not characteristic of any stage or degree of anoxia in these experiments.

The fact that the volume of the tidal air drops more rapidly and more markedly than the rate in the after-period indicates that the main factor which was active during the anoxia in increasing the depth of the respiration fades rapidly, while those which are increasing the rate persist longer. Lowered carbon dioxide and its sequelae may be a factor in producing a decreased depth and thus allow the pulmonary proprioceptive reflex to predominate. However, the increased rate in the chemoreceptor deafferented dog (4) in the presence of anoxia and a high carbon dioxide tension indicates that other influences are affecting the rate.

The inference may be drawn from these observations that the principal effect of the chemoreceptor reflex, as well as the chemical stimulation of the centers, is to increase the depth of the respiration, and that the rate increase, in anoxemia, is chiefly the result of other influences. The behavior of the dog with denervated chemoreceptors, reported in another paper (4), leads us to believe that the marked increases in the rate are mainly a manifestation of an anoxic depression of the central respiratory cells. Hence, the increase in rate becomes evident sooner and more markedly the lower the arterial oxygen tension.

A striking feature of these experiments is the greater tolerance of the dog than of man to low tensions of inspired oxygen. The dogs, without exception, first became unconscious, within about five minutes, at 5 per cent of inspired oxygen; thereafter they exhibited occasional periods of consciousness. This corresponds

to an altitude of about 30,000 feet. Comparable figures for man are not available, but, to choose the highest published figures, 23,000 to 25,000 feet would appear to be the altitude limit for consciousness for unacclimatized man (7).

A reason for the apparently greater resistance to anoxia of the dog, considering only the respiratory adjustments, may be partly a greater effectiveness of their chemoreceptors in increasing their ventilation. In a few experiments which we have performed on man, breathing low concentrations of oxygen, by the same methods used for the dog, although a variable degree of hyperpnea was produced in different individuals, the respiratory minute volume was always comparatively less than in the dog. The higher centers of the dog may be more resistant to anoxia than those of man; a comparison could only be made on the basis of equivalent circulating tensions of oxygen.

That some human subjects may tolerate as low oxygen tensions in the inspired air and behave qualitatively like the dog is indicated in the experiments of Horvath, Dill and Corwin (6) on dementia praecox patients. The subjects inspired 4.2, 5.2 or 6 per cent oxygen for periods ranging from 3 to 16 minutes, 6 to 21 minutes and 4 to 15 minutes respectively. Unconsciousness rarely occurred at 6 per cent, but was frequent below this concentration. The authors state that hyperpnea was frequently observed in the preliminary periods. This may possibly have contributed to the ability of the subjects to withstand such low inspired oxygen tensions for so long a time.

It may be that, with a lesser degree of respiratory adjustment in man suddenly exposed to anoxemia, the cortical and medullary centers are so quickly affected by the anoxia and the associated emotional disturbances, that the secondary factors of respiratory adjustment, demonstrable in the dog, do not have an opportunity to develop.

It must be emphasized that the dogs used in these experiments were trained normal unanesthetized animals. They were exposed suddenly and unvaryingly to a given concentration of oxygen during the anoxic period. The reactions of the animals were surprisingly uniform and characteristic at the different tensions of inspired oxygen. We have refrained from comparing our results with those of other experiments not conforming to these criteria.

#### SUMMARY

1. Normal, unanesthetized dogs, trained to breathe through a mask and valves, were subjected to nine levels of anoxia, varying from 16 per cent to 3 per cent of oxygen in the inspired air.

2. The characteristics of the respiratory minute volume, tidal air and rate, before, during and after the anoxia, are presented and discussed.

3. The data reveal that, during anoxemia, a chemical factor, other than carbon dioxide, as well as reflex stimulation from the aortic and carotid receptors is responsible for the respiratory response.

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# THE RESPIRATORY RESPONSES TO ANOXEMIA OF UNANESTHETIZED DOGS WITH CHRONICALLY DENERVATED AORTIC AND CAROTID CHEMORECEPTORS AND THEIR CAUSES<sup>1</sup>

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It is now generally believed, from evidence obtained in the anesthetized and unanesthetized dog, that the hyperpnea in response to anoxemia is reflexly initiated from chemoreceptors located in the carotid and aortic bodies. Evidence (1), supported by data presented in this paper, leaves no doubt that the unanesthetized dog chronically deprived of these chemoreceptors by operation may decrease the minute volume of the respiration when it breathes gas mixtures low in oxygen tension. This effect has been interpreted to be evidence that anoxia exerts a purely depressant action upon the respiratory centers and that without the chemoreceptors the animal is defenseless against anoxemia and death rapidly ensues. The question at issue is whether the admittedly immediate reflex response from the chemoreceptors is the sole mechanism which can produce a stimulation of respiration in response to anoxemia in the intact animal, or whether secondary factors may arise in a prolonged exposure which stimulate the respiration in spite of an undoubted central depression. No unequivocal evidence is at hand that anoxemia stimulates the respiration in an animal deprived of reflexes from the chemoreceptors in the carotid and aortic areas. The literature reveals no thorough systematic investigation in the unanesthetized animal with denervated chemoreceptors, which would be calculated to settle this question. Reports of delayed anoxic respiratory stimulation in unanesthetized denervated dogs (2, 3) have been so timidly presented or qualified that they are included by reviewers among the negative results. The secondary stimulation which Bouckaert, Heymans and Samaan (3) observed in the unanesthetized dog with chemoreceptors denervated was attributed by them to "psychomotor reactions" ("as a consequence of the primary inhibition") which "are depressed or suppressed by narcotics, anesthetics or decerebration."

Lack of space precludes an adequate discussion of the literature on this subject here. The majority of the evidence obtained on anesthetized animals has been interpreted as revealing little or no stimulation of respiration by anoxemia in animals with denervated carotid and aortic areas (4). However, it should be pointed out that the existing evidence of a profound effect of anesthesia and narcosis upon the respiratory centers (5) renders negative evidence obtained under these conditions of questionable value, an objection first raised by Dechar-

<sup>1</sup> Presented in preliminary form before the Physiological Society of Philadelphia. Dec. 15, 1942. (Proc. Phys. Soc. Phila. Am. J. M. Sc. 205: 311, 1943.)

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neux (6) and by Dautrebande (7). Their evidence led them to conclude that the unanesthetized dog with chronically denervated carotid and aortic chemoreceptors, unlike the anesthetized denervated dog, still responded to anoxemia with increased volume of respiration due to a direct effect upon the respiratory center. Moyer and Beecher (8) have noted that dogs, in which the cervical vagi had been severed and the carotid regions denervated, when under light anesthesia responded to low concentrations of inspired oxygen with a hyperpnea, which was reduced or abolished by slight deepening of the anesthesia. These investigators preferred to assume that the respiratory stimulation observed by them is due to excitation of the respiratory center, possibly by cellular acidity, and is not secondary to the occasional association of signs of generalized motor stimulation.

The experiments herein reported were initiated as a result of evidence presented in another paper (9), obtained upon trained normal unanesthetized dogs, which gives a clear indication of a secondary anoxemic hyperpnea which may be superimposed upon and additive to the initial response originating from the aortic and carotid bodies. To determine the cause of this secondary stimulation it became necessary to determine whether a similar response occurred in the absence of reflexes from the chemoreceptors. The data presented in this paper show that it does.

**METHODS.** The three dogs with their aortic and carotid chemoreceptors denervated were the same animals used in the experiments reported by Watt, Dumke and Comroe (1). The details of the operative procedures have been published (1). The operation preserved one recurrent laryngeal nerve (right), one abdominal vagus trunk (left) and a few fibers from the pressure receptors situated in the brachiocephalic artery, but it interrupted all fibers from the aortic bodies. The dogs showed the same lack of immediate respiratory stimulation on inhalation of low percentages of oxygen and the same lack of respiratory stimulation following injection of sodium cyanide as when used by these investigators. Hence, so far as these tests for completeness of chemoreceptor denervation can show, there was no evidence of nerve regeneration over a period of many months.<sup>3</sup>

The dogs were trained to lie quietly on their backs with only light restraint. Elsewhere we have described the apparatus (10) employed for administering the low oxygen mixtures or air to the dogs, and the technique (9) of registering and analyzing the respiratory records to obtain the minute volume, the volume of the tidal air and the rate of the respiration. The accuracy of the oxygen concentration was plus or minus 0.3 per cent.

The experiments consisted of a control fore-period of six minutes during which the dogs breathed atmospheric air either from a tube leading to the outdoor air or from the room. This was followed by the experimental period of twenty minutes or more during which the dogs inspired a constant concentration of the low oxygen gas mixture. This was usually followed by an after-period of ten minutes during which time the dogs again breathed atmospheric air.

Although the experiments were performed under uniform basal conditions of

<sup>3</sup> We are deeply indebted to Drs. Carl Schmidt and Julius Comroe for the generous loan of these dogs.

food, activity and environmental temperature there was considerable day to day variation in rate, depth and minute volume of the respiration in the control period; when these varied too greatly from the average the experiment was discontinued.

The effects upon the respiration of breathing gas mixtures low in oxygen were always calculated in terms of the percentage deviation from the room air control values obtained during that particular observation. Inasmuch as no significant qualitative differences were found in the response of the three dogs, the data obtained at any given oxygen percentage of the inspired air have been pooled.

Deafferented dogs are much less resistant to anoxia than are normal dogs. The latter can breathe 4 per cent of oxygen for an hour or more without failure (9), but the three deafferented dogs failed at ten, nine and eight per cent respectively. In this respect the deafferented dog more nearly resembles man in susceptibility to low oxygen inhalations than does the normal dog possessed of reflexes from the chemoreceptors. Failure consisted of an acute and intense dyspnea, violent struggles, vomiting, loss of consciousness and stoppage of respiration, differing only from the reaction in the normal dog in that the onset is more acute and, in these particular animals, a greater predominance of retching movements. Restoration of respiration after stoppage was always prompt when artificial respiration with room air was instituted; heart action continued throughout. In the experiments reported here the dogs were never carried to the point of failure. At an oxygen concentration of but one per cent above that at which they may fail the dogs were able to breathe the low oxygen mixture throughout the experimental period of twenty minutes or more with only an occasional period of restlessness. Usually, at the lowest oxygen percentages there was no restlessness at all. Reactions resembling convulsions never occurred.

The pulse rate was recorded at about two minute intervals, since it was found that an abrupt slowing of heart rate always preceded an impending failure.

**EXPERIMENTAL RESULTS.** The results of a typical experiment in which a deafferented dog breathed 12 per cent of oxygen are graphically shown in figure 1.

In a minute or less after the dog begins to breathe the low oxygen the minute volume of the respiration decreases from the control level until it reaches a minimum and then increases. When the dog again breathes atmospheric air, the minute volume of the respiration increases still further. Each of these events will be described separately.

*Period of anoxic respiratory depression.* The changes in the minute volume, the tidal air and the rate of respiration during the period of depression, at different percentages of inspired oxygen, are shown in table 1. The minute during which the volume of the expired air was least was chosen as a measure of the degree of the depression.

As the percentage of the inspired oxygen is reduced, the minute volume of the respiration is depressed from the control level by 15 to 28 per cent during the minute of maximum depression. The degree of depression does not increase with increasing anoxia but is minimal at the highest and lowest percentages of oxygen, between which a point of maximal depression might be statistically established.

At the time of maximum depression the volume of the tidal air falls below the control level and with increasing anoxia it declines to a level about one-third less than the control.

The respiratory rate, on the other hand, during the minute of maximum depression, is only slightly and insignificantly lower than its control value at the higher

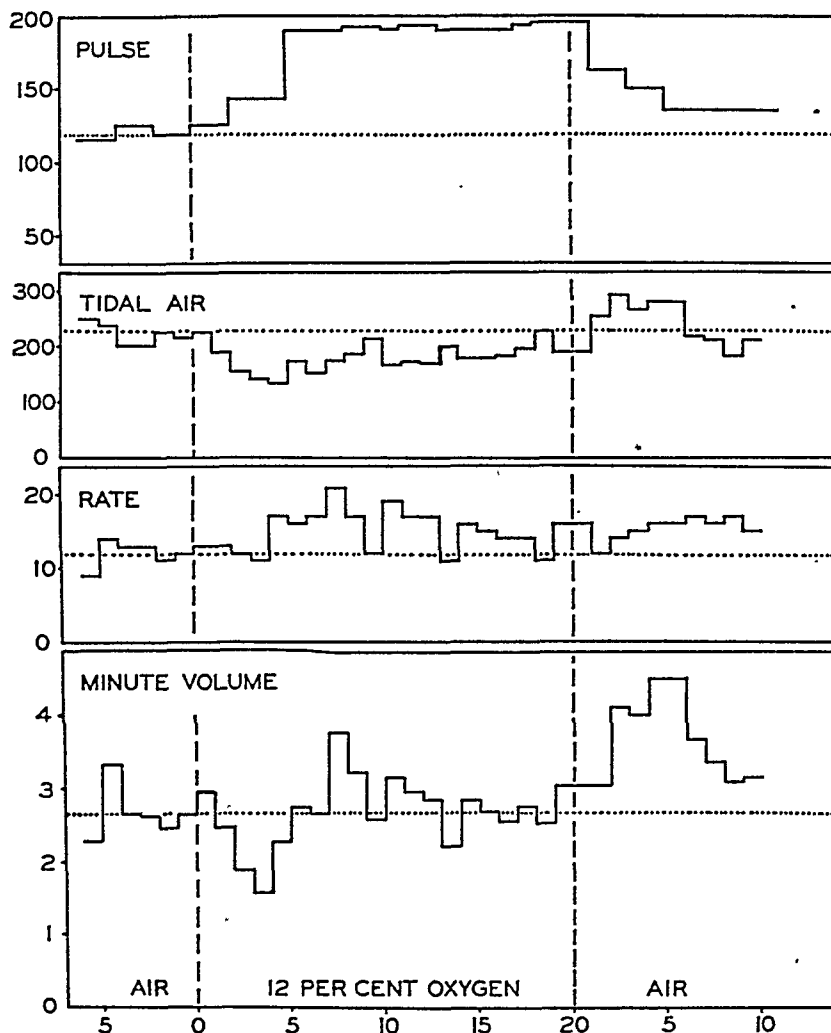


Fig. 1. The effect of anoxia on dog M. Abscissae: time in minutes. Ordinates: minute volume, liters per minute; rate, respirations per minute; tidal air, milliliter per respiration; pulse, beats per minute.

oxygen percentages, but as the anoxia is increased the rate rises, until at the lowest percentage of inspired oxygen it is 20 per cent greater than the control.

In the last column of table 1, the time from the beginning of the period of anoxia to the minute of maximum depression is compiled. It is evident that the maximum depression occurs progressively earlier with increasing anoxia. At 18 per cent of inspired oxygen it appears on the average in about eight minutes, but at 9 per cent it occurs at the third minute.

*Period of anoxic respiratory stimulation.* The depression is always transitory, and immediately following the point of maximum depression the minute volume



increases steeply and steadily until a plateau is reached (fig. 1), usually within the first ten minutes of the anoxic period. This increase is brought about largely by an increase in the rate of the respiration, but the tidal air also increases and approaches its control value. During the last ten minutes of a twenty minute period of anoxia, and for a much longer time in more prolonged experiments, the minute volume of the respiration remains relatively constant.

TABLE 1

*Characteristics of the respiration during the minute of maximum depression. Average percentage deviation of the minute volume, tidal air and respiratory rate from the average values during the control period. The time of maximum depression is the mean time in minutes from the beginning of the anoxia*

% O <sub>2</sub>	MINUTE VOLUME		TIDAL AIR		RATE		TIME MAX. DEPRESSION	
	No. of obs.	% Deviation from control $\pm$ stand. dev.	No. of obs.	% deviation from control $\pm$ st. dev.	No. of obs.	% Deviation from control $\pm$ stand. dev.	No. of obs.	Mean minutes $\pm$ stand. dev.
18	7	-15.2 $\pm$ 13.5	8	-5.8 $\pm$ 7.1	8	-8.8 $\pm$ 13.1	7	7.6 $\pm$ 4.6
16	15	-21.7 $\pm$ 14.4	15	-18.4 $\pm$ 14.4	15	-2.6 $\pm$ 11.7	15	6.8 $\pm$ 2.8
14	16	-28.4 $\pm$ 17.8	16	-19.9 $\pm$ 15.4	16	-5.9 $\pm$ 10.8	16	5.6 $\pm$ 1.7
12	17	-20.4 $\pm$ 13.4	17	-28.3 $\pm$ 12.1	17	+0.2 $\pm$ 13.7	17	3.6 $\pm$ 1.1
11	14	-27.3 $\pm$ 10.5	14	-32.0 $\pm$ 9.3	14	+10.7 $\pm$ 12.1	14	4.1 $\pm$ 1.7
10	10	-22.0 $\pm$ 9.3	11	-34.1 $\pm$ 15.9	11	+11.1 $\pm$ 11.1	10	3.4 $\pm$ 0.8
9	10	-15.0 $\pm$ 13.5	10	-29.1 $\pm$ 7.6	10	+20.1 $\pm$ 11.8	10	2.9 $\pm$ 0.7

TABLE 2

*Characteristics of the respiration during the last ten minutes of anoxic stimulation. Average percentage deviation of the minute volume, tidal air and respiratory rate from the average values during the control period*

% O <sub>2</sub>	MINUTE VOLUME		TIDAL AIR		RATE	
	No. of obs.	Means $\pm$ stand. dev.	No. of obs.	Means $\pm$ stand. dev.	No. of obs.	Means $\pm$ stand. dev.
18	8	-6.9 $\pm$ 14.8	8	+1.8 $\pm$ 7.1	8	-7.8 $\pm$ 11.3
16	15	+3.3 $\pm$ 27.0	15	-3.0 $\pm$ 10.1	15	+7.0 $\pm$ 27.4
14	16	+7.8 $\pm$ 19.2	16	-6.3 $\pm$ 7.1	16	+9.1 $\pm$ 18.5
12	16	+14.2 $\pm$ 31.8	16	-10.5 $\pm$ 10.6	16	+26.7 $\pm$ 35.8
11	14	+16.9 $\pm$ 27.2	14	-18.4 $\pm$ 8.7	14	+47.5 $\pm$ 38.6
10	11	+36.1 $\pm$ 56.1	11	-14.8 $\pm$ 9.6	11	+69.3 $\pm$ 77.8
9	10	+54.8 $\pm$ 57.1	10	-15.7 $\pm$ 5.7	10	+78.1 $\pm$ 58.7

The changes in the minute volume, the tidal air and the rate of the respiration during the period of anoxic stimulation are presented in table 2.

At 18 per cent of inspired oxygen the average minute volume during the period of anoxic stimulation, while greater than that at the minute of maximum depression, is slightly less than the control. As the inspired oxygen percentage decreases the minute volume of the respiration becomes progressively higher than the control, until at the lowest oxygen percentage the minute volume is about 55

per cent above the control. The greatness of the standard deviations in table 2 is caused by the skewness of the deviations, and not by the occurrence of cases in which the minute volume remained below the control.

The tidal air, except perhaps at 18 per cent of inspired oxygen, is slightly less than during the control period but it is by no means shallow, being at its least four-fifths or more of the control.

The rate of the respiration increases with increasing anoxia; at its maximum the rate is  $78.1 \pm 58.7$  per cent above the control values. Thus, a dog with a normal respiratory rate of 15 would increase its rate at most to about 30 per minute.

Although the period of anoxic stimulation has been prolonged for an hour or more, in many experiments, the dogs showed no signs of respiratory, cardiac or any other deterioration. Therefore, the respiration must have maintained an adequate if not an increased alveolar ventilation, and it definitely cannot be described as rapid shallow breathing with its usual unfavorable connotation.

TABLE 3

*Characteristics of the respiration during the minute of highest minute volume in the after-period. Average percentage deviation of the minute volume, tidal air and respiratory rate from the average values during the control period*

% O <sub>2</sub>	NO. OF OBS.	MINUTE VOLUME	TIDAL AIR	RATE
		Means $\pm$ stand dev.	Means $\pm$ stand dev.	Means $\pm$ stand dev.
18	5	+8.8 $\pm$ 12.3	+5.6 $\pm$ 8.3	0.0 $\pm$ 0.0
16	9	+14.0 $\pm$ 18.6	+9.7 $\pm$ 12.7	+3.7 $\pm$ 10.2
14	10	+31.5 $\pm$ 16.2	+20.1 $\pm$ 13.2	+5.4 $\pm$ 6.0
12	10	+43.1 $\pm$ 22.1	+23.4 $\pm$ 13.5	+10.9 $\pm$ 12.8
11	8	+36.6 $\pm$ 26.1	+25.0 $\pm$ 32.8	+13.3 $\pm$ 24.6
10	6	+23.2 $\pm$ 17.7	+10.3 $\pm$ 19.2	+11.8 $\pm$ 11.9
9	6	+46.8 $\pm$ 46.9	+10.7 $\pm$ 17.0	+34.3 $\pm$ 45.6

*Post-anoxic period.* When the dogs again breathe air, after breathing the low oxygen mixture, characteristic changes occur which in their relation to the events in the previous periods are typically shown in figure 1. In this experiment, after a few breaths of air, the minute volume of respiration rises very steeply for about five minutes, to reach a maximum which is greater than that in the preceding period of anoxic stimulation and then slowly falls, but it does not reach the control values at the end of ten minutes.

The characteristics of the respiration during the minute in which the highest volume was attained, for the different concentrations of inspired oxygen, are presented in table 3.

The average maximum minute volume exceeds the control level, and the difference tends to become greater as the preceding anoxia increases. The latter tendency is statistically significant at inspired oxygen concentrations of 14, 12 and 11 per cent as compared to 18 and 16 per cent. The maximum minute volume of the post-anoxic period is also greater than that of the anoxic period,

as is indicated by a comparison of these values in tables 2 and 3. The difference is statistically very significant at oxygen concentrations of 14 and 12 per cent.

The tidal air during the minute of maximum minute volume has risen significantly from a value below that of the control during the anoxic period (tables 1 and 2) to one above the control values. The post-anoxic increase in the tidal air appears to be maximal after the dogs breathed oxygen concentrations of 14, 12 or 11 per cent.

The respiratory rate decreases from the level attained during the anoxic stimulation. However, at the minute of maximum post-anoxic stimulation the rate is still somewhat above the control values. Its height above the control is greater the lower the oxygen concentration breathed.

In contrast to the anoxic stimulation, the increase of the minute volume in the after-period is produced mainly by an immediate deepening of the tidal air.

A typical picture of the pulse rate is shown in figure 1. It rises steeply and remains at a high level throughout the anoxic period. During the period of respiratory stimulation it usually rises to a still higher level. A prompt fall toward the control values follows when the dog again breathes air. A sharp fall in the pulse rate during anoxemia is a danger signal. This never occurred at the concentrations of oxygen used in the experiments presented in this paper.

**DISCUSSION.** The respiration as expressed in the minute volume, the tidal air and the rate is the resultant of all the influences converging on the respiratory centers.

The evidence presented in this paper proves that anoxemia causes central stimulation in the unanesthetized dog, chronically deprived of carotid and aortic chemoreceptor control. The data fully confirm the observation of Watt, Dumke and Comroe (1) that low oxygen inhalation by these dogs initially causes depression of the central respiratory mechanism. However, the depression of the minute volume of the respiration is transitory; it is not proportional to the degree of anoxia and it is followed by a prolonged elevation of the minute volume.

An adequate explanation of these findings is that the respiration in the anoxic deafferented dog is the resultant of two antagonistic factors, one a central depression, predominantly conspicuous early in the period of anoxia, and the other a central stimulation which later becomes dominant. Both of these effects increase with increasing anoxia. It is most probable that the central depression lasts as long as the anoxia, and that it is proportional to the degree of the anoxia. The reason that the minute volume does not proportionally reflect the central depression is that the anoxia also produces a concurrent stimulation of the centers which becomes sufficiently powerful to cut off the expression of the central depression and to raise and to maintain the minute volume at or above the level of the control, despite the persistence of a degree of central depression.

According to this view central depression and stimulation are coexistent, hence the maximal points of anoxic depression and stimulation of the respiration are only minimal measures of the true amount of depression and stimulation present. In both cases stimulation and depression add algebraically, and the minute volume observed represents their sum. Further evidence to be presented in a

later paper supports this view. If the anoxic stimulation is delayed, as it is in a deafferented polycythemic dog (11), the depression may be so profound that at an inspired oxygen as high as 14 per cent the dog may stop breathing for as long as a minute or more. On the other hand, if the stimulation is hastened, as it is in a deafferented anemic dog (11), the depression of the minute volume is almost entirely abolished.

The evidence that stimulation is present is that the depression is invariably transitory, that following the depression the minute volume rises to or above the control level, and that during the after-period there is a further elevation of the minute volume while the depression is rapidly diminishing. The evidence in this paper proves that both central depression and stimulation exists. On the supposition that they are both proportional to the degree of central anoxia, the characteristics of the respiration in these dogs can be explained. The proposed explanation is presented diagrammatically in figure 2.

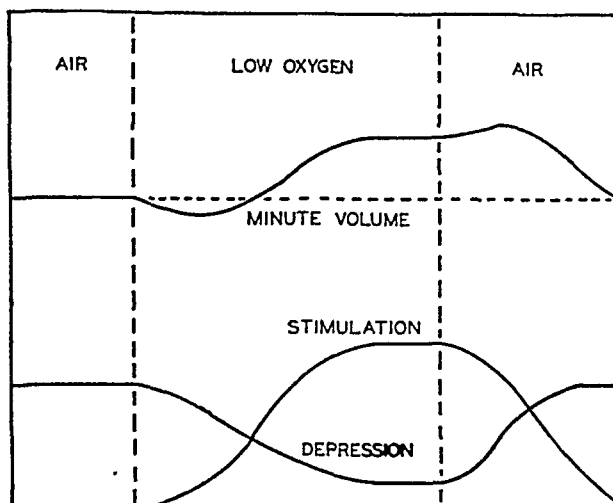


Fig. 2. Diagrammatic representation of the minute volume curve as the resultant of simultaneous stimulation and depression.

From the beginning of the anoxia the central depression increases until, in all probability, it would reach a level sufficiently low to cause a complete cessation of respiration were there no stimulation present. But at the time the depression is at a maximum a stimulation caused by the anoxia is rapidly increasing to an intensity great enough to overcome the anoxic depression. The resulting respiration is the sum of the depression and the stimulation. This accounts for the fact that the depression is always transitory. It also accounts for the fact that the depression of the minute volume is not greater as the anoxia becomes greater, for increasing stimulation not only cancels the increasing depression, but it may also diminish it, since the resultant increased alveolar ventilation, by increasing the tension of oxygen in the blood will reduce the central anoxia, which apparently relieves the central depression more quickly than it removes the stimulant. With increasing anoxia the stimulation increases the more rapidly and therefore the central depression is overcome sooner. This accounts for the observation

that the maximum depression in the minute volume of the respiration appears earlier the greater the anoxia.

The greatly increased minute volume of the respiration when the dogs are permitted to breathe air after a period of anoxia we regard as the strongest proof that stimulation is present. The respiratory centers recover from the anoxic depression, and the stimulation decays. It is clear that the recovery from depression occurs sooner than the decay of the stimulation, for upon resumption of breathing of air the minute volume rapidly rises even though it was above the control level during the preceding anoxic period. Moyer and Beecher (8) observed a post-anoxic hyperpnea greater than during the anoxemia, even in anesthetized dogs with denervated chemoreceptors. The degree to which the respiration was stimulated varied inversely with the depth of the anesthesia. The augmented respiration in most dogs was preceded by an immediate post-anoxic depression. This did not occur in our unanesthetized animals. They state that the post-anoxic hyperpnea is probably related to accumulation of acid products during the anoxia.

The origin, nature and mode of action of the stimulation of the respiration of the chemoreceptor deafferented dog during anoxemia is of considerable importance. Experiments designed to study this problem will be presented in another paper (11). Certain predictions may be made on the basis of the data in this paper. The rate of the production of the stimulant varies directly with the degree of the anoxemia. The relatively slow rise and slow decay of the stimulation suggests that it is chemical in nature. This is in striking contrast with the nervous stimulation in dogs with the chemoreceptors intact, for the nervous stimulation begins and ends within thirty seconds of the beginning and ending of the anoxia. The chemical stimulus is also discernible in dogs with intact chemoreceptors; it is superimposed upon the reflex stimulation and produces a residual effect following the removal of the nervous stimulation (9).

It is most unlikely that the stimulation is merely the effect of the accumulation of carbon dioxide during the depression of the minute volume. In many experiments, particularly at the lowest oxygen percentages, the depression is so small that there could be little accumulation of carbon dioxide, yet the stimulation is very great. Also, at the low oxygen percentages, the rate begins to rise in the first minute, before the minute volume has fallen enough to allow carbon dioxide to accumulate, and at the point of maximum depression it is well above the control rate. Once the stimulation of the minute volume has occurred it is maintained throughout the rest of the period of anoxia; minute volumes well above the control have been maintained for as long as an hour. If the stimulation had been due only to carbon dioxide, the excess of carbon dioxide would have been blown off early in the course of the stimulation, and the minute volume should have fallen, which it does not do. Finally, the increased ventilation during the anoxic stimulation is accomplished by a greater increase in rate than in the depth of the respiration. Carbon dioxide, administered to these dogs during anoxia, increased the depth and had little effect on the existing rate.

The respiration of the dog with deafferented chemoreceptors, during an an-

oxemia, is characterized by a rapid rate to a greater extent than in the normal dog (9). The anoxic depression of the minute volume, at the higher concentrations of inspired oxygen, is due to a decrease in the volume of the tidal air; the decrease in the rate is insignificant. At the lower concentrations of oxygen, on the other hand, the respiration is both rapid and shallow. This increased rate is an evidence that stimulation is present. It would, therefore, appear that an increased rate is characteristic of the respiration when the centers, depressed by anoxia, are stimulated, both in the dog with intact and deafferented chemoreceptors. The initial recovery from the respiratory depression of anoxia in the deafferented dog is due to an increase in rate, but the volume of the tidal air increases gradually until in many experiments it reaches or slightly exceeds the control values. This recovery in the depth of the respirations may be due either to increase in the stimulant or to its greater effectiveness in increasing the tidal air, because of some relief of the central depression, due to an improved oxygen tension of the blood. The latter explanation would seem to fit best the events in the after-period. Upon resumption of air breathing, the respiration of the deafferented dog promptly increases in depth and the rate diminishes, due to a removal of the central depression and persistence of the stimulation. The rate may remain above the control level for some time. This may be a reflection of a residue of central anoxic depression.

The fact that the anoxia can be prolonged for an hour or more with no evidence of deterioration in the respiration, circulation or well-being of the dog is sufficient proof that the respiration is effective in maintaining an adequate alveolar ventilation. This conclusion is supported by data on the oxygen content of the blood to be reported in a later paper. The progressive improvement in the depth of the respiration and thus in the effective alveolar ventilation during the anoxia indicates a greater and a more effective stimulation with time.

#### CONCLUSIONS

1. Anoxemia causes stimulation as well as depression of the central respiratory mechanism in the unanesthetized dog, chronically deprived of carotid and aortic chemoreceptor control.

2. Depression of the minute volume of the respiration during anoxia in such a dog is transitory; it is not proportional to the degree of anoxia, and it is followed by a stimulation which causes a prolonged elevation of the minute volume, and an effective alveolar ventilation.

3. The stimulation of the respiration induced by anoxia persists and the minute volume is still further increased when the dogs are again permitted to breathe air.

4. It is postulated that the respiration in the anoxemic deafferented dog is the resultant of two antagonistic coexistent factors, a central depression and a central stimulation. The minute volume of the respiration represents the algebraic sum of these effects. Although the central depression may vary in degree with the anoxia, the stimulation also increases with increasing anoxia, hence the depression of the minute volume is not greater as the anoxia becomes greater,

for increasing stimulation cancels increasing depression. Recovery from the depression is more rapid than decay of stimulation, hence the greatly increased minute volume of respiration when air breathing is resumed.

5. The evidence points strongly to a central chemical stimulation. Reasons are presented which preclude carbon dioxide as the responsible agent.

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# EFFECT OF ORGAN EXTRACTS AND THEIR FRACTIONS ON ACETYLCHOLINE SYNTHESIS<sup>1</sup>

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In the presence of human blood serum (1) and of spinal fluid (2) more acetylcholine is synthesized by fresh frog brain than in the presence of Ringer's solution. In the following the effect of tissue extracts and various fractions thereof on the acetylcholine synthesis was ascertained.

**METHOD.** The effect of the various extracts and their fractions on the synthesis of acetylcholine was ascertained by a modified method of Quastel, Tennenbaum, and Wheatley (1, 3). One or two cubic centimeters of the fluid to be tested or 1 cc. of Ringer's solution containing the substances to be tested in various concentrations (pH 7.4), 100 mgm. minced fresh frog brain, 3 mgm. physostigmine salicylate, and 1 to 2 cc. Ringer's solution were shaken and incubated aerobically for 4 hours at 37°C. After incubation the amounts of acetylcholine synthesized were assayed biologically on the sensitized rectus abdominis muscle of the frog.

**Calculation.** The amounts of acetylcholine synthesized were calculated by subtracting from the acetylcholine content of the incubated mixtures the acetylcholine content of identical non-incubated mixtures. The amount of acetylcholine synthesized in the control mixtures containing only brain, physostigmine, and Ringer's solution was taken as 100 per cent. The acetylcholine content of the mixtures containing the various substances used was expressed as a percentage of the control. All results deviating from 100 per cent by more than twice the square root of the sum of the squares of the standard error of the controls and the standard error of the experiments ( $2\sqrt{\text{S.E.}_{\text{control}}^2 + \text{S.E.}_{\text{experiments}}^2}$ ) were considered significant deviations.

**RESULTS.** *Effect of Saline Extracts of Different Organs on Acetylcholine Synthesis.* Cats were killed by intravenous injection of air. Immediately after the occurrence of death the heart, striated muscle, lung, and liver were removed and washed free of blood. The organs were placed in Ringer's solution two times their volume and were finely triturated by a Waring blender. The mixtures were shaken for two hours at room temperature. Afterwards, the mixtures were centrifuged, the pH of the supernatant solution corrected to 7.4 (when necessary), and the effect of 2 cc. of the supernatant fluid on the acetylcholine synthesis was ascertained.

The results are given in table 1. The extracts increased the acetylcholine synthesis to either the same extent as did human serum (whole) or somewhat more.

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*Effect of Red Blood Cells on Acetylcholine Synthesis.* To ascertain whether the formed elements of human blood have an effect on acetylcholine synthesis similar to that of serum, 12 cc. blood were collected from human subjects into test-tubes containing 3 cc. of a 20 per cent sodium citrate solution. This mixture was centrifuged for 20 minutes at low speed and the red blood cells were washed three times with an isotonic saline solution. Afterwards, the red blood cells were hemolyzed by suspension in 6 cc. of distilled water. The samples were centrifuged, an aliquot of the supernatant fluid was removed, and salts of

TABLE 1

*Effect of serum, serum filtrates and tissue extracts on acetylcholine synthesis*

AGENTS	AMOUNT OF ACETYLCHOLINE SYNTHESIZED IN PER CENT OF CONTROL FOLLOWED BY THE STANDARD ERROR OF THE MEAN					
	Extract obtained by treatment with:					
	Ringer's solution		50 per cent alcohol and 50 per cent ether		Ether	
	No. of expts.	Amt. of acetylcholine	No. of expts.	Amt. of acetylcholine	No. of expts.	Amt. of acetylcholine
Serum (whole).....	20	204 $\pm$ 2.2	10	71 $\pm$ 2.5		
Serum dialysate.....	5	166 $\pm$ 4.5				
Serum ultrafiltrate....	10	162 $\pm$ 2.5				
Filtrate of boiled serum.....	27	150 $\pm$ 1.4				
Demineralized filtrate of boiled serum.....	3	172 $\pm$ 2.2				
Hemolysate of red blood cells.....	10	190 $\pm$ 3.2	10	74 $\pm$ 1.5		
Residue of hemolysate of red blood cells.....	10	222 $\pm$ 3.5	10	72 $\pm$ 0.5		
Muscle.....	10	235 $\pm$ 2.9				
Heart.....	10	175 $\pm$ 3.5				
Lymph gland.....					10	100 $\pm$ 1.9
Thyroid.....					10	112 $\pm$ 2.3
Subcutaneous fat.....					10	105 $\pm$ 1.4
Lung.....	10	202 $\pm$ 2.6			10	104 $\pm$ 1.8
Salivary gland.....					10	105 $\pm$ 2.1
Thymus.....					10	63 $\pm$ 0.8
Pancreas.....			10	52 $\pm$ 2.6	10	54 $\pm$ 1.3
Liver.....	10	198 $\pm$ 3.3	10	44 $\pm$ 2.2		

Ringer's solution were added. The effect of 1 cc. of this solution on the synthesis of acetylcholine was ascertained by the method described above.

The amount of acetylcholine synthesized in the presence of the hemolysate was similar to the amount obtained in the presence of serum (table 1).

In another series of experiments  $\frac{1}{2}$  cc. of the residue of the red blood cells obtained by centrifugation was suspended in the frog brain mixture and the amount of acetylcholine synthesized was ascertained. The amount of acetylcholine synthesized was similar to that obtained in the presence of some tissue extracts (table 1).

*Effect of Fractions of Serum and Tissue Extracts on Acetylcholine Synthesis. Dialysate.* Serum was dialyzed through cellophane membrane by means of a glass vessel divided by a cellophane membrane into two compartments. Serum was placed into one compartment and Ringer's solution into the other one. The vessel was shaken vigorously at room temperature for 4 hours. Two cubic centimeters of the dialysate were added to the mixture of frog brain and the amounts of acetylcholine synthesized were determined. An average of 66 per cent more acetylcholine was synthesized in the presence of the dialysate than in the control mixtures (table 1). Some substances contained in the serum that increase acetylcholine synthesis were, therefore, dialyzable.

*Ultrafiltrate.* Ultrafiltrate of serum was prepared by forcing the serum through a cellophane membrane under 40 lb./sq. in. pressure. In the presence of 1 cc. of this ultrafiltrate the synthesis of acetylcholine increased on the average by 62 per cent (table 1).

*Filtrate of boiled serum.* Blood serum was diluted with an equal volume of water, boiled at pH 7, and filtered. The filtrate was re-concentrated to its original volume by evaporation. On the average 50 per cent more acetylcholine was synthesized in the presence of this boiled serum than in the control mixtures (table 1). Many of the substances responsible for the increase of the acetylcholine synthesis were, therefore, heat stable and filtrable.

*Non-filtrable fractions.* There is a difference between the amounts of acetylcholine synthesized in the presence of whole serum and that of the filtrate of serum. To ascertain whether this difference is due to some of the non-filtrable substances contained in the serum or whether the difference is only an artefact induced during dialyzation, the effect of serum proteins and five plasma fractions supplied by Dr. E. J. Cohn (4) were also tested as to their effects on acetylcholine synthesis.

The results are given in table 2. Hemoglobin, blood albumin, and the five plasma fractions increased the amount of acetylcholine synthesized. (The fractions were: I contained approximately 60 per cent fibrinogen and a mixture of the other plasma proteins; II + III contained about 40 per cent  $\gamma$ -globulin, about 40 per cent  $\beta$ -globulin, and a mixture of plasma lipids (cholesterol, phosphatides, and carotenoids); IV-1 contained  $\alpha$ -globulins and plasma lipids (cholesterol and phosphatides); IV-3, 4 contained  $\alpha$ -globulin,  $\beta$ -globulin, traces of lipids, and some carbohydrate; V contained albumin and 3 per cent  $\alpha$ -globulin.)

The increase of the amount of acetylcholine synthesized in the presence of the non-filtrable substances was similar, suggesting that the effect on the synthesis was due to some of the amino acid components of the substances used. (Amino acids are found to increase acetylcholine synthesis (5).)

*Demineralized filtrate of boiled serum.* Since most of the inorganic ions contained in the serum will pass through the cellophane membrane, and since many inorganic ions may increase acetylcholine synthesis (6, 7), the filtrate of boiled serum was demineralized by passing it through ion-exchange columns (Amberlite 1R-100 and 1R-4). The demineralized ultrafiltrate increased the amount of acetylcholine synthesized somewhat more than did the original filtrate (table

1), indicating that the organic substances contained in the demineralized filtrate of boiled serum increase the acetylcholine synthesis.

*Ether extracts.* To ascertain whether the filtrate of serum contained only substances that increase acetylcholine synthesis or whether it was a mixture of both potentiator and depressor substances, the filtrate of boiled serum was further fractionated. The filtrate of serum was extracted with ether by means of a continuous ether extractor for 24 hours. The ether was evaporated to dryness and the residue was suspended in Ringer's solution corresponding to the original volume of serum filtrate. This extract decreased the amount of acetylcholine synthesized by 17 per cent ( $83 \pm 4.0$ , average of 5 experiments), indicating that the serum contained depressor substances as well as substances that increase the amount of acetylcholine synthesized. To ascertain whether or not the existence of the depressor agents was due to some artefacts induced during the extraction with ether, the effect of the residue on acetylcholine synthesis was also tested.

TABLE 2  
*Effect of serum proteins on acetylcholine synthesis*

SUBSTANCE	AMOUNTS OF ACETYLCHOLINE SYNTHESIZED IN PER CENT OF CONTROL FOLLOWED BY THE STANDARD ERROR OF THE MEAN. (EACH VALUE REPRESENTS THE AVERAGE OF 10 SEPARATE EXPERIMENTS)			
	Amounts of the substances added to 100 mgm. frog brain in mgm.:			
	3	0.3	0.003	0.0003
Blood albumin	$144 \pm 2.1$	$130 \pm 1.9$	$119 \pm 1.3$	$113 \pm 1.6$
Hemoglobin	$130 \pm 1.7$	$135 \pm 2.1$	$127 \pm 2.3$	$109 \pm 0.9$
Plasma fractions:				
I	$140 \pm 2.9$	$141 \pm 2.5$	$126 \pm 1.6$	$116 \pm 0.5$
II + III	$127 \pm 2.1$	$122 \pm 2.6$	$122 \pm 2.4$	$104 \pm 0.8$
IV-1	$139 \pm 3.3$	$130 \pm 2.7$	$119 \pm 2.2$	$114 \pm 1.1$
IV-3, 4	$131 \pm 2.8$	$122 \pm 1.7$	$110 \pm 1.0$	$107 \pm 0.9$
V	$142 \pm 2.4$	$143 \pm 2.1$	$132 \pm 2.0$	$117 \pm 0.7$

The residue increased the amount of acetylcholine synthesized by 33 per cent more than did the filtrate of serum ( $183 \pm 6.0$ , average of 2 experiments), indicating that the original ultrafiltrate contained depressor agents.

Ether extracts were prepared also by more crude methods.

Serum hemolysate obtained from washed red blood cells, and the residue of red blood cells obtained after hemolysis by centrifugation were shaken at room temperature with a mixture containing 50 per cent absolute alcohol and 50 per cent ether for 4 hours. Liver and pancreas of cat were triturated in a Waring blender with two volumes of a mixture containing 50 per cent absolute alcohol and 50 per cent ether. The mixtures were shaken vigorously at room temperature for 4 hours. Afterwards, the mixtures were centrifuged and the alcohol-ether layer evaporated to dryness. Three milligrams of the residue were added to the frog brain preparation (pH at 7.4) and the amounts of acetylcholine synthesized determined as described above.

All the ether extracts decreased somewhat the synthesis of acetylcholine (table 1).

Thymus, pancreas, lymph gland, thyroid, salivary gland, lung and subcutaneous fat were also extracted by a method described by Novinski (8, 9). Fresh minced tissues were immersed in acetone for 6 hours, the acetone discarded, and the tissues were then extracted in a continuous ether extractor for 24 hours. The ether layer was evaporated to dryness. Three milligram samples of the dry extracts were suspended in the frog brain mixture to test their effect on acetylcholine synthesis.

Extracts of thymus and pancreas contained agents that depressed the synthesis of acetylcholine, the other organs did not contain the depressor agents in amounts sufficient to be demonstrable with the method used (table 1).

*Fractions of the ether extracts (fatty acids, non-volatile aldehydes and ketones).* Concentrates of the fatty acid content of serum and tissue extracts and concentrates of the non-volatile aldehydes and ketones were prepared by the following method: filtrate of boiled serum and deproteinized saline extract of liver were acidified to pH 2 and extracted with ether by vigorous stirring with a flattened rod moved in a test-tube. The ether extract was shaken vigorously with Ringer's solution at an alkaline pH by means of the flattened rod. This alkaline saline extract was separated from the ether layer and freed from traces of ether by heating to 36°C. and passing air through it. The ether residue was evaporated to dryness. The alkaline saline extract is a concentrate of the original fatty acid content of the serum filtrate and the liver extract, the ether residue is a concentrate of the non-volatile aldehydes and ketones. The effects of the alkaline saline extracts (pH corrected to 7.4), the ether residue, and the residue of acidified serum filtrate and liver extract after ether extraction (pH corrected to 7.4) on acetylcholine synthesis were tested.

The alkaline saline extract (concentrate of fatty acids) decreased the amount of acetylcholine synthesized (table 3, column 3), indicating that some of the fatty acids contained in the original serum filtrate and liver extract decreased acetylcholine synthesis. It was previously shown that in the presence of unsaturated and some of the higher fatty acids less acetylcholine is synthesized than in the control mixtures (10).

The amounts of acetylcholine synthesized in the presence of the ether residue (concentrate of non-volatile aldehydes and ketones) and the control mixtures were similar (table 3, column 4), indicating that (1) the aldehydes and ketones contained in the ether residue did not significantly modify the amount of acetylcholine synthesized, and (2) agents, contained in the original filtrate and extract of liver, that increased acetylcholine synthesis did not pass in the ether extract. The possibility that the potentiator and depressor agents counteracted each other's effect cannot, however, be excluded.

The original filtrate of serum lost its ability to increase acetylcholine synthesis by acidification and extraction with ether (table 3, column 2).

*Fractionation of ether extracts (cephalin, phospholipids, cholesterol and cholesterol esters.* The brain of animals killed by injection of air into the vein was triturated

in a Waring blender together with two volumes of a mixture of 50 per cent absolute alcohol and 50 per cent ether. Afterwards, the brain suspension was shaken for 4 hours at room temperature. The suspension was then centrifuged and the supernatant ether-alcohol layer evaporated to dryness. The lipoids were isolated and purified from the dry mass by a method described by Chargraff, Bancroft, and Stanley-Brown (11).

Various amounts of lecithin, cephalin, and the fraction containing cholesterol and cholesterol esters were added to the frog brain preparation and the amounts of acetylcholine synthesized determined. The fraction containing cholesterol

TABLE 3  
*Effect of fractions of serum filtrate and liver extract on acetylcholine synthesis*

AGENTS	AMOUNTS OF ACETYLCHOLINE SYNTHESIZED IN PER CENT OF CONTROL FOLLOWED BY THE STANDARD ERROR OF THE MEAN. (EACH VALUE REPRESENTS THE AVERAGE OF 10 SEPARATE EXPERIMENTS)			
	Boiled filtrate	Residue of acidified filtrate after ether extraction	Alkalinized saline extract of ether extract from acidified boiled filtrate	Ether extract residue
Serum.....	150 $\pm$ 1.5	78 $\pm$ 2.8	73 $\pm$ 2.2	90 $\pm$ 1.3
Liver (saline extract).....	156 $\pm$ 2.7		74 $\pm$ 2.5	91 $\pm$ 1.0

TABLE 4  
*Effect of brain extracts on acetylcholine synthesis*

SUBSTANCE	AMOUNTS OF ACETYLCHOLINE SYNTHESIZED IN PER CENT OF CONTROL FOLLOWED BY THE STANDARD ERROR OF THE MEAN. (EACH VALUE REPRESENTS THE AVERAGE OF TEN SEPARATE EXPERIMENTS)		
	Amounts of the substances added to 100 mgm. frog brain in mgm.:		
	3	0.3	0.03
Cephalin.....	79 $\pm$ 2.0	85 $\pm$ 2.7	88 $\pm$ 2.2
Lecithin.....	148 $\pm$ 1.9	128 $\pm$ 2.1	120 $\pm$ 1.3
Cholesterol and cholesterol esters.....	95 $\pm$ 2.2	97 $\pm$ 1.5	98 $\pm$ 0.4

and cholesterol esters did not modify the amounts of acetylcholine synthesized, cephalin decreased the synthesis and lecithin increased it (table 4).

#### SUMMARY AND CONCLUSION

The results presented above indicate that blood serum and animal tissues contain both substances that increase acetylcholine synthesis and substances that decrease it. Human blood serum and water extracts of various animal tissues contain substances that increase acetylcholine synthesis. These substances are in part non-dialyzable (proteins, lipoproteins) and in part dialyzable, many of them heat stable and organic.

Ether extracts of serum and various tissues contain factors that decrease

acetylcholine synthesis. For some of the depressor effects fatty acids are responsible.

The authors wish to express their gratitude to Dr. Charles O. Warren for the ultrafiltrate of serum, the deproteinized serum filtrates, the demineralized serum filtrate, and the first ether extract of serum filtrate and to Dr. Edwin J. Cohn for the five plasma fractions.

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# VARIATIONS IN PLASMA VOLUME AND HEMATOCRIT VALUES FOLLOWING HEMORRHAGE IN UNANESTHETIZED NORMAL, SPLENECTOMIZED AND SYMPATHECTOMIZED DOGS<sup>1</sup>

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It has been reported (17, 19, 23, 29) that when the hematocrit value is lowered by bleeding, the red cell volume calculated from the plasma volume and hematocrit reading does not agree with the volume expected on the basis of the volume of red cells removed. Stead and Ebert (29) believe that this lack of agreement is the result of the unequal distribution of red cells in the various parts of the circulatory system (10, 28).

In the present study the changes in hematocrit values and plasma volume which occur in normal dogs immediately and for several days after hemorrhage have been compared with those which are found under the same conditions in splenectomized and sympathectomized animals. It was hoped that the variations in the response to hemorrhage shown by splenectomized and sympathectomized animals might aid in interpreting the results obtained on normal dogs.

**METHODS.** Sixteen experiments were carried out upon 12 unanesthetized dogs. Of these 6 were normal, 4 were splenectomized and 3 were sympathectomized. One normal animal was subsequently splenectomized and appears in 2 groups. Sympathectomies were performed in 2 stages, as described by Cannon et al. (5). The time between the first operation and the experiment varied from 39 to 44 days, which is a shorter period than that required for the regrowth of nerve fibers to the adrenal glands (16). At least 5 months elapsed before the normal and splenectomized animals which were bled twice were used for the second experiment.

Experiments were carried out 18 to 20 hours after the last feeding. During the control period mean blood pressure was measured by arterial puncture (Hg manometer), heart rate was counted and rectal temperature and body weight were determined. Blood samples for dye estimations, red cell counts, reticulocyte counts and hematocrit readings were drawn without stasis from the jugular vein or femoral artery. The femoral artery was cannulated and over a period which varied from 2 to 106 minutes in different animals, 25 to 45 per cent of the estimated blood volume was removed. In 12 of the 16 experiments the bleeding time was less than 12 minutes. The blood was prevented from clotting by a mixture of ammonium and potassium oxalate (22). The above determinations were repeated 1 hour after the hemorrhage and on each of the succeeding days until the hematocrit reading had reached its minimal value.

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Plasma volume was estimated with the blue dye, T-1824 (11, 12, 13). The plasma protein concentrations were determined refractometrically (21).

TABLE 1

*Effects of hemorrhage upon plasma volume in unanesthetized normal, splenectomized and sympathectomized dogs*

CONTROL			HEMORRHAGE			EX- PECTED	POST-HEMORRHAGE				
Dog	B.V.	P.V.	B.V.	P.V.	B.V.	P.V.	1 hr.	Diff. bet.	Maximum		
							P.V.	P.V. found & exp. .	P.V.	P.V.	P.V.
	cc.	cc.	cc.	cc.	% Control	cc.	cc.	% Control	cc.	days after hem.	% Contro
Normal											
1	1466	683	420	188	28.6	495	544	7.1	854	2	124.9
2	2004	1040	752	388	37.4	652	710	5.5	1255	1	120.4
2*	1978	989	560	314	28.3	675	707	3.3	1215	2	122.9
3	1019	685	436	293	42.8	392	390	-0.3	680	3	99.4
4	821	486	320	188	39.0	298	386	18.1	503	2	103.8
5	1428	780	469	261	32.9	519	580	8.0	880	4	112.8
6	1057	573	404	231	38.2	342	376	6.0	615	3	107.3
Splenec- tomized								6.8			113.1
								8.0†			115.4†
	1292	764	324	200	25.0	564	686	16.0	829	4	108.2
	1508	985	427	282	28.3	703	879	17.8	1072	2	109.0
	822	466	303	177	36.9	289	277	-2.6	449	1	96.4
	1175	720	322	218	27.4	502	693	26.6	859	2	119.1
	1593	1003	512	332	32.1	671	818	14.6	1041	2	103.8
	747	454	242	149	32.4	305	362	12.5	487	1	107.1
Sympa- thec- tomized								14.2			107.3
								17.5‡			109.4‡
	825	500	370	232	44.8	268	330	11.5	608	3	121.6
	834	505	310	191	37.2	314	425	22.1	592	2	117.0
	1262	754	459	267	36.3	487	530	5.7	778	3	103.1
								13.1			113.9

B.V. = Blood volume (includes white blood cell volume).

P.V. = Plasma volume.

\* Second bleeding.

† Average excluding dog 3.

‡ Average excluding dog 8 (first bleeding).

Hematocrit readings were made in Wintrobe tubes on heparinized blood which had been centrifuged at 3000 r.p.m. for  $\frac{1}{2}$  hour. The hematocrit values were



corrected for plasma trapping (13). Blood and red cell volumes were calculated from plasma volume and hematocrit readings (see 25). Oxalated blood was diluted with 3 per cent sodium chloride in certified pipettes, and the red blood cells were counted in the usual way. Reticulocytes were

TABLE 2  
Effects of hemorrhage upon red cell volume in unanesthetized normal, splenectomized and sympathectomized dogs

Effects of hemorrhage upon red cell volume in unanesthetized normal, splenectomized and the red blood volume in sympathectomized dogs									
CONTROL		HEMORRHAGE		EXPECTED	POST-HEMORRHAGE				Diff. bet. RBC. vol. found & exp.
Dog	R.B.C. vol.	R.B.C. vol.		R.B.C. vol.	1 hr.	Diff. bet. RBC. vol. found & exp.	Minimum		
	cc.	cc.	% Control	cc.	cc.	% Control	cc.	days after hem.	% Control
Normal	765	227	29.7	538	484	-7.1	343	3	-25.5
	952	360	37.9	592	674	8.7	435	1	-16.4
	967	240	24.8	727	663	-6.6	555	1	-17.8
	318	137	43.1	181	222	12.9	137	3	-13.8
	318	126	39.7	192	248	17.7	129	2	-19.7
	635	204	32.1	431	416	-2.3	329	4	-16.0
5	477	169	35.5	308	295	-2.7	178	4	-27.1
Splenectomized						2.9			-19.5
	506	120	23.7	386	345	-8.1	340	2	-9.1
	503	139	27.6	364	380	3.1	370	3	1.1
	346	123	35.6	223	163	-17.2	140	2	-23.9
	445	101	22.7	344	271	-16.5	258	2	-19.3
	571	174	30.5	397	359	-6.7	340	1	-10.0
	284	91	31.9	193	180	-4.8	149	3	-15.6
						-8.4			-12.8
Sympathectomized						-10.5†			-15.6†
	317	134	42.3	183	204	6.7	159	2	-7.5
	312	116	37.2	196	214	5.8	154	1	-13.4
	497	188	37.9	309	345	7.3	278	1	-6.1
						6.6			-9.0

RBC. = Red blood corpuscle.

\* Second bleeding.

† Average excluding dog 7 (first bleeding).

stained by adding a drop of 3 per cent aqueous cresyl blue solution to a drop of blood containing a crystal of potassium oxalate. This was smeared on a glass slide, counterstained with Wright's stain and 500 cells were counted, the reticulocytes being estimated as a percentage of the red blood cells.

The CO capacity was determined on the blood of 2 dogs using the method described by Roughton et al. (26).

RESULTS. *Normal dogs.* Seven experiments were carried out on 6 normal dogs (tables 1, 2 and 3) which were bled from 28 to 43 per cent of their control blood volumes. In 5 of the 6 animals the plasma volume, 1 hour after hemor-

TABLE 3  
*Effects of hemorrhage upon the total number of red blood corpuscles*

CONTROL				HEMOR- RHAGE	EX- PECTED	POST-HEMORRHAGE				
Dog	RBC.	BV.	RBC.	RBC.	RBC.	1 hr. RBC.	Diff. bet. RBC. found & exp.	Minimum RBC.		Diff. bet. RBC. found & exp.
	<i>mil/cu. mm.</i>	<i>cc.</i>	<i>tril.</i>	<i>tril.</i>	<i>tril.</i>	<i>tril.</i>	% Control	<i>tril</i>	<i>days after hem.</i>	% Control
Normal										
1	7.60	1466	11.12	3.28	7.84	6.95	-8.0	5.14	3	-24.2
2	7.34	2004	14.70	5.31	9.39	9.69	2.0	7.31	1	-14.1
2*	6.75	1978	13.35	3.70	9.65	9.01	-4.8	7.55	1	-15.7
3	4.78	1019	4.88	2.08	2.80	3.34	11.0	1.91	3	-18.2
4	5.52	821	4.53	1.76	2.77	3.54	17.0	1.86	2	-20.0
5	6.47	1428	9.25	2.99	6.26	6.38	1.3	4.87	4	-15.0
6	7.82	1057	8.27	2.87	5.40	4.91	-5.9	3.26	4	-25.9
							3.2			-19.0
Splenecto- mized										
1*	5.92	1292	7.65	1.81	5.84	5.33	-6.7	4.63	2	-15.8
7	4.78	1508	7.21	2.04	5.17	5.44	3.7	5.02	3	-2.1
8	5.25	822	4.32	1.71	2.61	2.04	-13.2	2.04	2	-13.2
9	5.26	1175	6.20	1.54	4.66	4.29	-5.9	3.38	2	-20.6
7*	5.78	1593	9.22	2.58	6.64	4.98	-18.0	4.54	1	-22.8
8*	5.02	747	3.74	1.43	2.31	2.45	3.7	2.20	3	-2.9
							-6.1			-12.9
Sympathec- tomized										
10	5.80	825	4.78	2.14	2.64	3.17	11.1	2.48	2	-3.4
11	4.52	834	3.76	1.40	2.36	2.91	14.6	2.03	1	-8.8
12	5.34	1262	6.75	2.54	4.21	4.74	7.8	3.96	1	-3.7
							11.2			-5.3

tril. = trillion.

\* Second bleeding.

rhage, was from 3 to 18 (average 8) per cent greater than was expected (control plasma volume minus plasma removed). During the succeeding 1 to 4 days the plasma volume increased rapidly until it was 4 to 25 (average 15) per cent above the control value. In dog 3 the plasma volume did not increase 1 hour after hemorrhage and 3 days later it was still less than the control value. This

animal was bled a larger fraction of the control blood volume than were the other dogs in the group.

The red cell volumes of the 7 normal dogs were greater 1 hour after hemorrhage in 3 animals and less in 2 animals than the value predicted (control red cell volume minus red cell volume removed). In 2 dogs the residual red cell volumes were equal to the calculated values (table 2). During the succeeding days all of the animals showed a decrease in the cell volume as compared with that present 1 hour after hemorrhage. On the day of the minimal hematocrit reading the cell volumes were 14 to 27 per cent less than the volumes expected at the end of

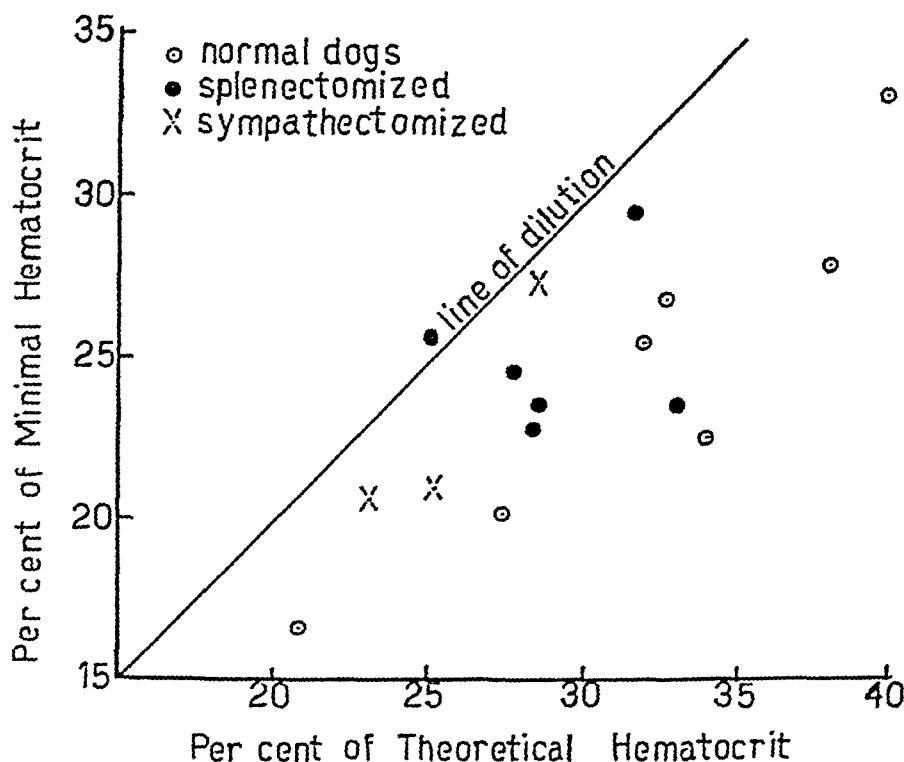


Fig. 1. The hematocrit on the day of the minimal hematocrit reading is plotted on the ordinate. On the abscissa is the theoretical hematocrit calculated from the red cell volume present in the body after hemorrhage (control red cell volume—red cell volume bled) and the plasma volume on the day of the minimal hematocrit. If red cells are neither added to nor removed from the circulation the values should fall on the line of dilution.

hemorrhage (table 2). Figure 1 shows that after hemorrhage the minimal hematocrit reading cannot be explained as the result of dilution.

The number of red blood cells in the animal was calculated from the red cell count and the blood volume. The variation in the number of red cells at various times after hemorrhage was in excellent agreement with the changes in red cell volume (table 3). Chronologically reticulocytosis followed the establishment of the minimal red cell volume.

When the hematocrit readings (range 23 to 45 per cent) were compared with the CO capacities (range 10 to 20 vols. per cent) on 14 samples of blood taken

from 2 dogs before and each day for several days after hemorrhage, an excellent linear correlation was found. According to these measurements the hematocrit value corrected for plasma trapping ( $\times 0.96$ ) and multiplied by  $0.439 \pm 0.00859$  is equal to the CO capacity of the blood.

One hour after hemorrhage the blood volume was 2 to 18 (average 5) per cent greater than the expected value (control volume minus blood removed) in 5 experiments and 0.2 to 2 (average 1) per cent less in 2 experiments. Although the volume increased during the succeeding 1 to 3 days, it did not exceed 96 per cent of the control value in any animal (average 87).

No significant variations in body weight or rectal temperature were observed. The post-hemorrhagic fall in blood pressure was followed by an immediate rapid rise, but the control value was not reached on the day of the bleeding. The initial decrease in heart rate that occurred a few minutes after the bleeding was followed by a rapid rise. The tachycardia persisted on the day after the hemorrhage, but returned to the control level on the following day.

*Splenectomized dogs.* Six experiments were carried out upon 4 splenectomized dogs (tables 1, 2 and 3). The animals were bled from 25 to 37 per cent of their blood volumes. One hour after hemorrhage the plasma volume in 5 of the 6 experiments was 13 to 27 (average 18) per cent greater than the value expected (control plasma volume minus plasma removed). During the succeeding 1 to 4 days the plasma volume increased until it was 4 to 19 (average 9) per cent above the control value. One hour after hemorrhage the plasma volume of dog 8 on the first bleeding was equal to the predicted value. In this animal, which was bled more than the other dogs in the group, the plasma volume did not rise above the control value during the next few days.

Except for the first bleeding on dog 7 the red cell volume averaged 11 per cent less than the value expected (control red cell volume minus red cells removed). In the different dogs the red cell volumes fell to a minimal value on either the first, second or third day after the bleeding. The minimal volume attained ranged from 9 to 24 (average 16) per cent lower than the value expected immediately after hemorrhage (table 2). Figure 1 shows that the minimal hematocrit reading can be explained on the basis of dilution in only one instance.

In 5 of the 6 experiments the blood volume was 6 to 13 (average 8) per cent greater than the predicted value (control minus the blood removed) one hour after the bleeding. During the succeeding 1 to 4 days the blood volume increased, but it did not exceed 97 per cent (average 90) of the control volume in any animal. In the first bleeding on dog 8, the volume failed to rise above 76 per cent of the pre-hemorrhagic value during the 4 days in which the animal was studied.

The post-hemorrhagic fall in blood pressure was followed by a slow rise which failed to attain the control value the day after bleeding. In all animals except the second bleeding on dog 7, the heart rate increased and remained above the control value for 2 days after the hemorrhage.

*Sympathectomized dogs.* Three sympathectomized dogs were bled from 36 to 45 per cent of their blood volumes. One hour after bleeding, the plasma vol-

ume was 6 to 22 (average 13) per cent greater than the volume expected (control plasma volume minus plasma removed). During the succeeding 2 or 3 days the plasma volume increased rapidly until it was 3 to 22 (average 14) per cent greater than the control values (table 1).

One hour after hemorrhage, the red cell volume was 6 to 7 per cent greater than the value expected (control red cell volume minus cell volume removed). In the following 1 or 2 days the volume of the red cells decreased until it was 6 to 13 (average 9) per cent less than the volume expected immediately after bleeding (table 2). Figure 1 shows that the minimal hematocrit reading cannot be explained solely on the basis of dilution.

The blood volume was 7 to 15 (average 11) per cent greater than the predicted value (control minus blood removed), one hour after hemorrhage. During the succeeding 2 or 3 days the blood volume increased until it averaged 90 per cent of the control value.

Forty to 67 per cent of the blood pressure loss was recovered on the day of the bleeding. The heart rate increased after hemorrhage in 2 of the 3 sympathectomized dogs, 18 and 34 beats per minute; it decreased 22 beats per minute in the third animal. Rectal temperature was depressed about 3°C by hemorrhage. No significant variations in body weight were observed.

**DISCUSSION.** The above experiments show that when normal, splenectomized or sympathectomized dogs are bled 25 to 45 per cent of their blood volumes, the decrease in red cell volume is greater than can be accounted for on the basis of the volume of red cells removed. That the change in hematocrit reading from which the change in red cell volume is calculated cannot be explained as the result of hemodilution is shown in figure 1. Similar observations have been made by bleeding splenectomized dogs (29) and normal dogs anesthetized with barbital (19). According to Ebert et al. (9) the red cell volume of professional blood donors who were bled 15 to 20 per cent of their blood volume, as determined from dye and hematocrit readings, agreed with the volume expected immediately after hemorrhage. However, after dilution had occurred and the hematocrit level had decreased, the red cell volume was less than the value predicted from the blood lost.

The apparent loss in red cells which occurs after hemorrhage has been explained by various investigators in different ways. Ebert and Stead (9) attributed the phenomenon to a shift in the amount of blood present in the small blood vessels. This explanation rests upon the finding that the red cells are not uniformly distributed throughout the vascular bed, the blood from the large vessels having a higher cell-plasma ratio than the blood contained in the minute vessels (28, 10, 18, 8, 14). Recent comparisons of the blood volume as measured with the dye and hematocrit reading with that estimated with carbon monoxide (3, 25) show a close agreement between the observed hematocrit and that calculated from the red cell volume and the blood volume suggesting that the amount of blood present in the small vessels must be small. Indeed, Bazett (4) calculates that at most only 13 per cent of the blood can be in the capillary bed. These observa-

tions indicate that the missing red cells cannot be accounted for by a redistribution of blood. The disappearance of red cells after bleeding sympathectomized dogs suggests that in these animals the loss of red cells is not related to differences in the neurogenic state of the peripheral circulation.

The decreased red cell volume could result from a reduction in the mean corpuscular volume. Anisocytosis has been noted between the second and thirteenth days after hemorrhage (1, 6). According to Adolph et al. (1) the small cells poor in hemoglobin are those recently released by the bone marrow. However, Naegeli (20) has suggested that the decreased diameter is compensated by an increased thickness of the cells. Since in the above experiments the average decrease in red cell volume or hematocrit is the same as the decrease in the total number of red blood cells calculated from the red cell count, no change in the mean corpuscular volume can have occurred. Moreover, the linear correlation between the hematocrit reading and the hemoglobin concentration as measured by the CO capacity indicates that the hemoglobin distribution in the cells is unchanged. Immature reticulocytes are not released until after the establishment of the minimum hematocrit reading and, therefore, do not influence the mean corpuscular volume.

The decrease in the hematocrit value which follows hemorrhage and which in the above experiments cannot be accounted for on the basis of dilution would occur if there were a decreased production or an increased destruction of red cells. The progressive decrease in hemoglobin which Seaman and Ponder (27) observed in man after various surgical operations was ascribed by them to the inhibition of hematopoiesis. Anisocytosis, observed by the author and others several days after hemorrhage, probably indicates a bone marrow release of cells which may be related to stimulation of blood forming tissues and the eventual delivery of immature cells to the circulation. According to Drinker et al. (7), immature cells cannot be washed out of the marrow and, therefore, their presence in the blood must represent a true stimulation of hematopoiesis. In connection with the destruction of cells, it should be noted that Hawkins et al. (15) have shown that red cell destruction is proportional to the number of circulating red cells. In agreement with this observation, the author has found in a few rough experiments that the output of urobilin on the day after hemorrhage is less than that determined during a comparable control period.

It is possible that after hemorrhage red cells disappear into certain regions of the vascular bed where they are withdrawn from the active circulation. The fact that the fall in hematocrit reading is shown by splenectomized dogs demonstrates that the spleen alone cannot be involved, and that cell segregation must take place in extra-splenic reservoirs (2, 24). This interpretation is not accepted by Lawson and Rehm (19) who believe that the apparent loss of red cells is the result of an error inherent in the dye and hematocrit method of measuring blood volume. Reasons for believing that the dye and hematocrit method is a reliable technique for measuring blood volume have been given in a recent publication from this laboratory (25).

## SUMMARY

1. Unanesthetized normal, splenectomized and sympathectomized dogs were bled from 25 to 45 per cent of their blood volumes (dye and hematocrit readings). One hour after hemorrhage the red cell volume was greater in some animals and smaller in others (table 2) than the value predicted (control red cell volume minus red cell volume removed). During the succeeding 1 to 4 days the red cell volume of all but one splenectomized dog decreased to a minimum which averaged 9 per cent less than the value expected in the sympathectomized dogs. The corresponding values were 13 per cent for the splenectomized and 20 per cent for the normal dogs.

2. Dilution alone cannot account for the decreased hematocrit value (fig. 1). The agreement between the reductions in red cell volume and in the total number of red blood cells indicates that no decrease in the mean corpuscular volume has occurred (tables 2 and 3). The linear correlation between the hematocrit reading and the hemoglobin concentration as measured by the carbon monoxide capacity shows that no change has occurred in the hemoglobin content of the red cell. Determinations of urobilin demonstrate no increased post-hemorrhagic destruction of red cells.

3. It is suggested that after hemorrhage red cells disappear into certain regions of the vascular bed where they are withdrawn from the active circulation.

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# LOCAL DIFFERENCES IN INTRATHORACIC PRESSURE AND THEIR RELATION TO CARDIAC FILLING PRESSURE IN THE DOG

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The present study is an outgrowth of what appeared to be conflicting observations on the circulatory changes occurring during pressure breathing (1, 2). Repeated observations have shown that a reduction in cardiac stroke output occurs during periods of elevated intrapulmonary pressure (8, 9, 1, 7, 15, 17, 12). This reduction in stroke output might conceivably be caused by the accompanying rise in intrathoracic pressure, increasing the resistance to the entrance of blood into the chest and thereby reducing venous return. Boyd and Patras (1) have demonstrated that the reduced stroke output is accompanied by a fall in the difference between right atrial and intrathoracic pressures. Boyd and Brookhart (2), however, failed to detect any significant change in this pressure difference in experiments in which no cardiometer was used. In the former instance, the ventricles were enclosed in a rigid-walled cardiometer, the chamber of which communicated with a small, closed pneumothorax. In the latter instance, the heart lay in its normal relations to the pericardium and lungs.

Two other possible causes of a reduction in cardiac stroke output during pressure breathing have occurred to us. *a.* Increased intrapulmonary pressure in an animal with a closed chest might increase pulmonary vascular resistance, reducing venous return to the left ventricle. *b.* Pressure breathing might produce an elevation of pressure above the general intrathoracic level, acting selectively upon some portion of the intrathoracic circulatory system. Neither of these possibilities has been given adequate consideration in the past. The present experiments indicate that intrathoracic pressure is not uniform throughout the chest, but is higher in the region of the heart. Evidence of the manner of production of such a local elevation of pressure is presented.

**METHODS.** The results to be presented were obtained from experiments on fifteen dogs anesthetized with morphine sulfate (4.0 mgm./kilo. SC) and barbital sodium (175-200 mgm./kilo. IV). The recordings varied from experiment to experiment depending upon the points under consideration. Concordant findings were obtained from a sufficient number of individual animals to convince us of their validity.

Optical pressure recordings were made using a glass-membrane manometer system which is a modification of that described by Green (3). The manometers are made from Pyrex tubing having an outside diameter of 1 cm. and a wall thickness of 1 mm. The membrane is blown into the side of the tubing rather than into the end. The manometer is then used in the upright position, thus

facilitating the detection and elimination of air bubbles. The manometer holder, with the sealed-in lens and threaded cap, is shown in sectional diagram in figure 1. Connections to vessels are made through lead tubing. The manometers may be used for recording gas or liquid pressures against atmospheric pressure, or as differential manometers recording pressure differences between liquids and gas.

Atrial pressure was recorded from a jugular sound passed in to the vicinity of

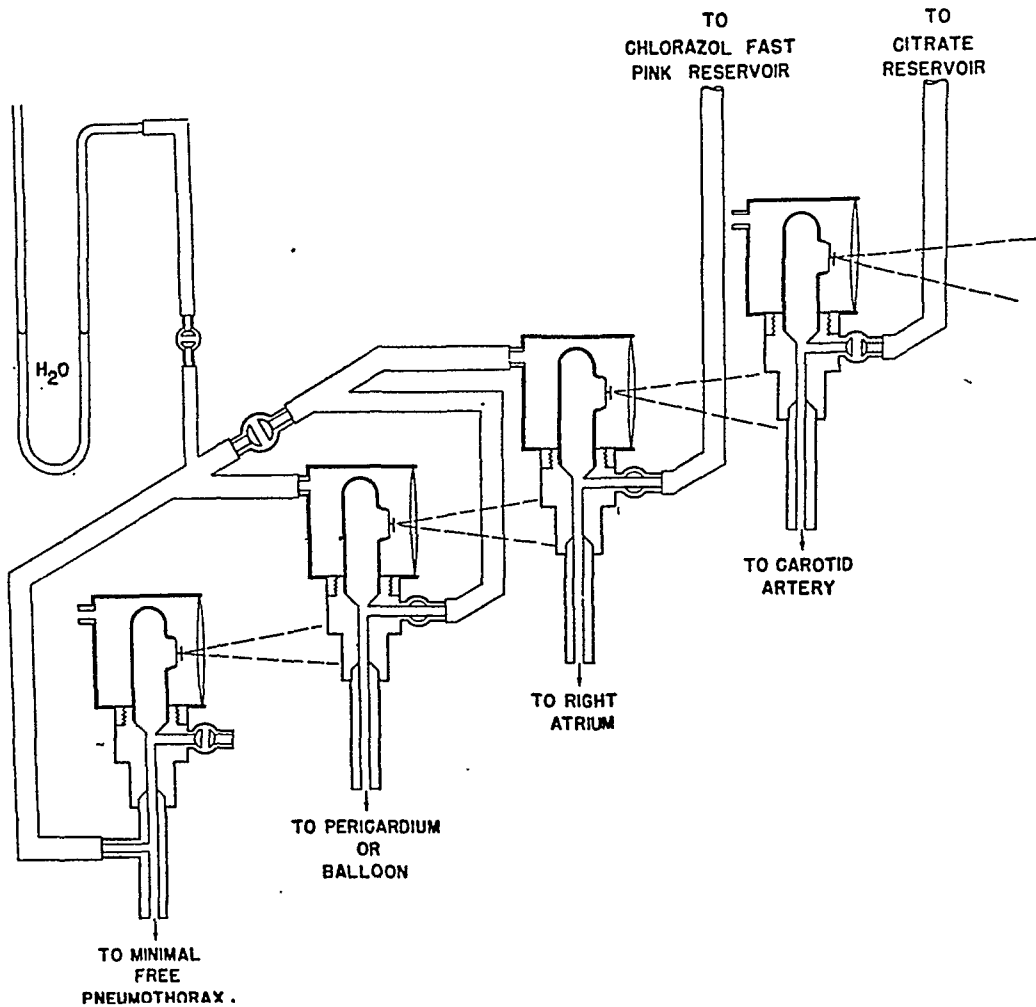


Fig. 1. Sectional diagram indicating the glass-membrane manometers, their holders, and the connections allowing for simple and differential pressure recordings. The water manometer shown at the left of the diagram was used as a convenient check on the level of intrathoracic pressure and was disconnected during recordings.

the right atrium, or by direct cannulation of the left auricle. Clotting was prevented by allowing a slow, continuous flow of chlorazol fast pink to enter the sound except during the periods of actual recording (14). By manipulation of the stopcocks shown in figure 1, atrial pressure could be recorded differentially against pressure in a pneumothorax bubble, or against the pressure in an air-pocket in some other portion of the chest. An attempt was made at the termination of each experiment to record a zero level for atrial pressure. With the chest

widely opened, and with the pericardium reflected to its extreme, a portion of the right atrium was cut away. It was hoped that the resting position of the manometer under these conditions would indicate a zero reference level. In some experiments this was obviously not the case. Apparently, shifts in the position of the heart due to the destruction of the ventral mediastinum and pericardium, or to the buoyancy of the lungs floating on blood accumulated in the chest, made it impossible to obtain reliable zero levels. For this reason, the calibrations of atrial pressure records shown in the figures are accurate insofar as they indicate the extent of the changes of pressure, but cannot be regarded reliably as indicating the true level of pressure.

Intrathoracic pressure was recorded from a trocar thrust between the ribs just dorsal to the caudal portion of the sternum. Intrathoracic pressure was varied by adjusting the size of the pneumothorax bubble surrounding the trocar. In some experiments, pressure between the lung and the lateral thoracic wall was recorded from a hemispherical balloon, 3.5 cm. in diameter, mounted on a shallow disc which was secured to the inner wall of the chest.

After numerous failures, a technique was developed which permitted accurate recording of pressure changes occurring between the lateral aspect of the pericardium and the adjacent lung. To attain accuracy it was necessary to avoid as completely as possible any distortion of the lung or interference with its free movement. Distortion was minimized by using a large, thin-walled balloon with a minimal air content, thus holding a small air bubble in place in a container which could conform most readily to the shape of the surrounding structures. Interference with the free movement of the lung was avoided by keeping the intrapleural portion of the transmission tube as short as possible. To this end, the connecting tube from the manometer was passed into the lower end of the esophagus. At its distal end it was joined to a small U-tube, one arm of which was passed through an aperture in the lateral wall of the esophagus and was held firmly with a purse-string. To this intrapleural arm of the U was attached a short length of nylon tubing bearing a segment of condom approximately 4 cm. in length. The nylon tube extended the full length of the balloon and had numerous holes cut in its sides throughout the length covered by the balloon. The distal end of the balloon was held in place by a stitch through the mediastinal pleura or pericardium. The balloon, when inflated in outside air, was capable of holding 30 ml. of air without a rise of pressure. Recordings of pressure in the balloon were routinely made with an air volume of 4-5 ml., pressure being recorded differentially against pressure in a small, closed pneumothorax.

Positive intrapulmonary pressure was applied through the tracheal cannula from a reservoir tank supplied with a continuous flow of compressed air. Pressure in the tank was adjustable by means of a variable exhaust port placed near the tracheal cannula. By using a three-way valve, the animal could be made to breathe air under atmospheric pressure, or to inspire compressed air from the tank and expire through the exhaust port. A pressure gauge was placed in the line near the tracheal cannula.

Entrance to the chest was gained in some experiments through a midline sternal incision. In other experiments, it was more convenient to carry out a subperiosteal rib resection on a segment of the fourth or fifth rib. Closure was accomplished with the help of cotton soaked in a thick solution of warm gelatin. The edges of the sternum, or the periosteal bed, were approximated and overlaid with gelatin-soaked cotton. The muscle and skin layers were then tightly closed over the cotton.

**RESULTS.** *The Existence of Local Variations in Intrathoracic Pressure. Simultaneous recordings of right and left effective filling pressures.* Previous investigations have not established firmly the nature of the changes of pulmonary vascular resistance during pressure breathing (10, 20, 5). If an increase in pulmonary vascular resistance developed during pressure breathing, right effective filling pressure might be sustained by back-pressure effects. Under these conditions, left effective filling pressure would be expected to diminish. In figure 2 pres-

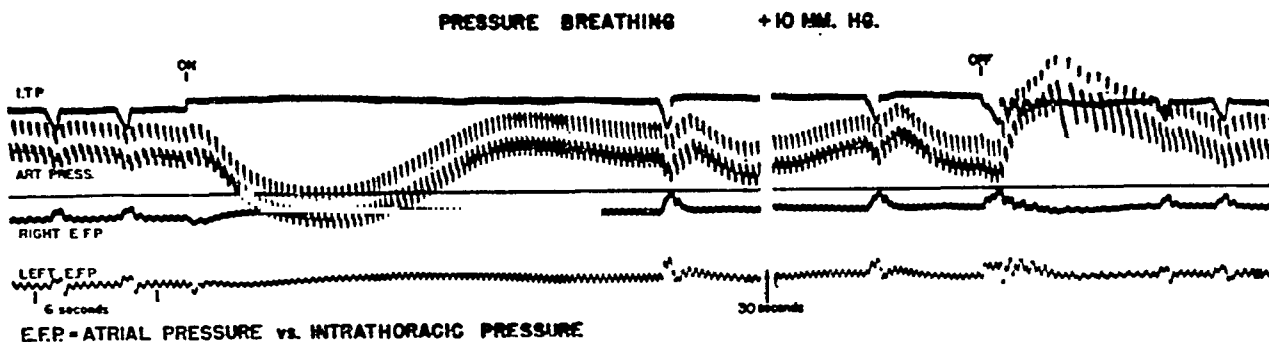


Fig. 2. The effects of increased intrapulmonary pressure (+10 mm. Hg) upon intrathoracic pressure (ITP), carotid arterial pressure (ART. PRESS.), and right and left effective filling pressures (EFP). Neither right nor left effective filling pressure show reductions which would be expected to occur if stroke volume were to fall as a result of reduced venous return.

ures in the right and left atria are recorded against pressure in a small, closed pneumothorax, after the technique usually employed in measuring effective filling pressure. The record indicates that neither right nor left effective filling pressures, recorded simultaneously during pressure breathing, shows any sustained reduction. Arterial pressure and pulse contour changes are typical of a reduction in stroke volume. Observations of the animals, and recordings of abdomino-thoracic pressure difference during pressure breathing lead us to the conclusion that the rise of effective filling pressures, occurring early on the right and later on the left side, is a result in part of an increase in expiratory abdominal tonus forcing blood into the thorax. The timing of the rise of left effective filling pressure suggests that the recovery of systemic arterial pressure is not entirely dependent upon systemic vasoconstriction.

*Extracardiac and intrathoracic pressure differences.* A localized increase in extracardiac pressure during pressure breathing would act to restrain cardiac filling even though the difference between atrial pressure and pressure in a free

pneumothorax bubble remained the same. Figure 3 is a record obtained during a single respiratory cycle in a vagotomized dog breathing air under atmospheric pressure. Pressure in a small pneumothorax bubble lying between the lung and the thoracic wall varies from 5 to 10 cm.  $H_2O$  below atmospheric pressure during the respiration. Pressure in a balloon between the lung and the lateral wall of the pericardium is recorded differentially against the pressure in the pneumothorax. As the record shows, pressure in the balloon is between 2 and 3 cm.  $H_2O$  higher than "intrathoracic pressure," and the difference becomes greater during the slow, deep inspiration of the vagotomized animal. The extent of the difference varied somewhat from animal to animal and was influenced by the character of the respiration. The direction of the difference, however, was always such as to indicate that extracardiac pressure, under the conditions of the experiments, was higher than pressure between the lung and thoracic wall.

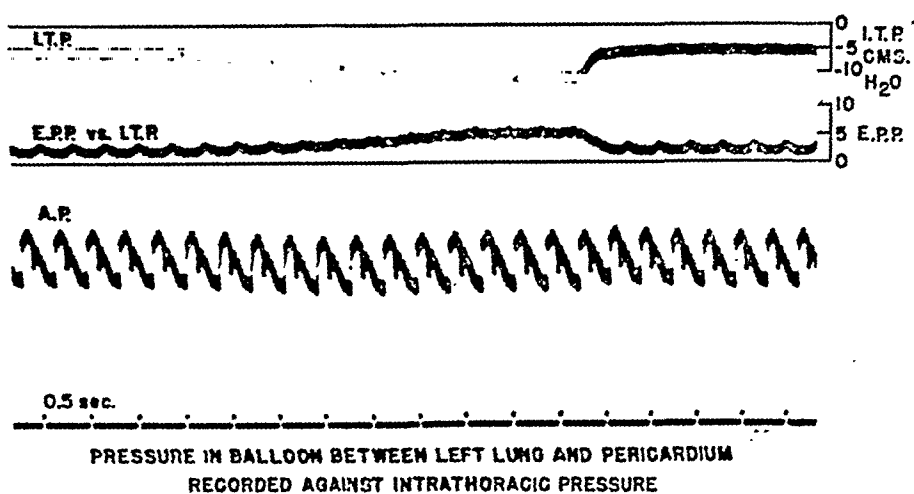


Fig. 3. Pressure in an extrapericardial balloon recorded differentially against pressure in a pneumothorax bubble during an inspiration in a vagotomized dog. Extrapericardial pressure—EPP; intrathoracic pressure—ITP; carotid arterial pressure—AP.

*Atrial and extracardiac pressure differences.* The effects of the elevation of extracardiac pressure on differential atrial pressure are indicated in figure 4. The two records of this figure were taken from the same animal within a few minutes of each other. The recording immediately above the time line shows that the difference between extracardiac and "intrathoracic" pressure becomes greater during pressure breathing. The influence of the elevation of extracardiac pressure may be seen by comparing the changes in right atrial pressure when recorded differentially against "intrathoracic" pressure (4 above) and against pressure in an extrapericardial balloon (4 below). In the former instance, atrial pressure suffers only a very slight and temporary reduction. In the latter instance, the reduction of atrial pressure is pronounced and is sustained throughout the period of application of pressure breathing. A similar elevation of extracardiac pressure, and a similar reduction of differential atrial pressure, occurs whether the balloon is on the right or the left side of the heart.

*The Cause of Local Variations in Intrathoracic Pressure.* Since the lungs and the heart are hollow, elastic viscera competing for space within the closed cavity of the thorax, we considered that pressure variations such as those described above might possibly result from local distortion of the elastic lung tissue surrounding the heart. This hypothesis was tested by producing distortion of the lung under controlled conditions. This was accomplished by the graded inflation of a hemispherical balloon mounted between the lateral surface of the lung and the wall of the chest. Pressure in the balloon at various air volumes was recorded differentially against pressure in a closed pneumothorax of minimal volume.

Results typical of such an experiment are presented in the graph of figure 5.

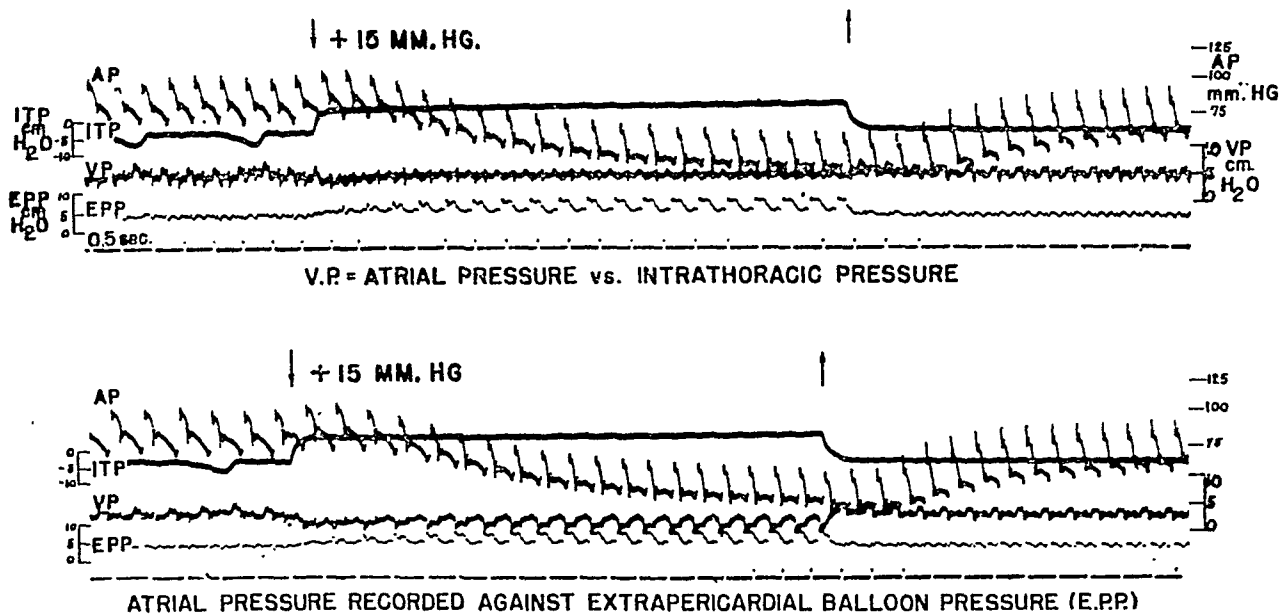


Fig. 4. Above: Atrial pressure recorded against pressure in a small closed pneumothorax during increased intrapulmonary pressure (+15 mm. Hg). Below: The same animal a few minutes later. Atrial pressure recorded against pressure in an extrapericardial balloon. Intrathoracic pressure—ITP; carotid arterial pressure—AP; differential atrial pressure—VP; differential extrapericardial pressure—EPP; time—0.5 sec.

This graph presents the relation between balloon volume and differential balloon pressure. The straight horizontal dashes indicate the extent of the pressure variations in the pneumothorax. As the figure shows, even with the balloon volume as low as 6 ml., pressure in the balloon was almost 3 cm. H<sub>2</sub>O higher than pressure in the pneumothorax bubble. As the balloon volume was increased, pressure in the balloon rose higher and higher above "intrathoracic" pressure. Inspiratory values were consistently higher than expiratory values. The rise in differential pressure during inspiration was greater than would have been predicted from the volume change occasioned by the inspiratory drop in general "intrathoracic" pressure. Thus, the differential pressure at the 14 ml. balloon volume rose 3 cm. H<sub>2</sub>O during inspiration, whereas the volume change in the

balloon was less than 0.2 ml. Tension in the wall of the balloon itself was not responsible for the elevation of balloon pressure above "intrathoracic" pressure. As indicated by the values obtained with the chest open and the lungs completely collapsed, the balloon wall was not subjected to tension until the balloon held between 14 and 15 ml. of air.

The influence of elastic tension of the lungs in the elevation of the balloon pressure was further investigated in experiments of the type described by figure 6. Balloon volume was held constant at 8 ml. and "intrathoracic" pressure was adjusted by varying the size of the pneumothorax bubble. Expiratory values of "intrathoracic" and differential pressures were related. As the lung tension

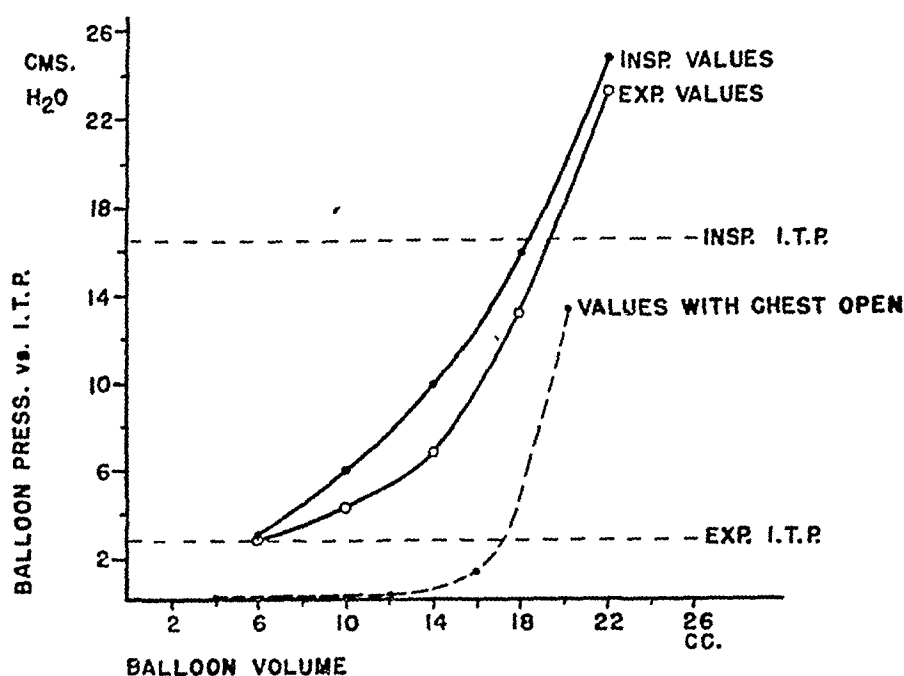


Fig. 5. Hemispherical balloon pressure recorded differentially against pressure in a small, closed pneumothorax and plotted in relation to various balloon volumes. The balloon lies between the lateral wall of the lung and the chest wall. The horizontal dashes indicate the extremes of variation in pressure in the small, closed pneumothorax (*I.T.P.*). The values obtained with the chest open and the lungs collapsed indicate that the balloon walls are not placed under tension until the balloon volume reaches 14 ml.

diminished with increasing degree of lung collapse, the pressure difference between the air in the balloon and the air in the pneumothorax also diminished. In some experiments, the curve deviated from its approach to zero as the collapse of the lungs became more extreme. In these instances it was seen *post mortem* that the collapse of the lungs had allowed the heart to fall laterally toward the side of the chest in which the balloon was mounted.

A similar relation of lung tension to pressure is seen to exist with reference to the elevation of extracardiac pressure above "intrathoracic" pressure. The records of figure 7 illustrate the changes of extracardiac pressure which occur during progressive collapse of the lungs. Pressure in a balloon lying between the

heart and the left lung is recorded differentially against pressure in a pneumothorax bubble. As the lung tension increases with inspiration, the differential pressure rises. As the lung tension is reduced by enlargement of the pneumothorax, differential pressure falls.

DISCUSSION. The factors responsible for the development of a subatmospheric pressure between the wall of the lung and the wall of the thorax have been analyzed in minute detail by Rohrer (16). Among the forces involved is that force which depends upon the elastic tension existing in the tissue of the lung. While tension in the wall of the lung may be directed in any plane, the tendency of the lung to retract from adjacent structures is dependent upon the tension

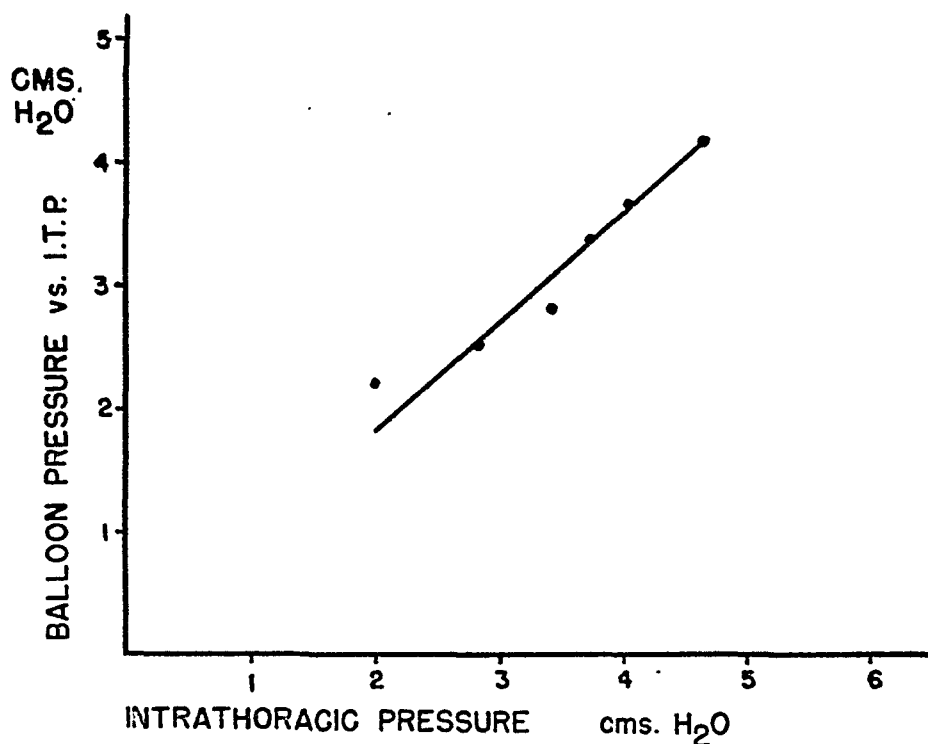


Fig. 6. Expiratory values of hemispherical balloon pressure recorded differentially against pressure in a small, closed pneumothorax and plotted in relation to various intrathoracic pressures. Balloon volume held constant at 8 ml.

vector which is directed inward normal to the surface of the lung. If this tension vector is uniform over the entire surface of the lung, it must then follow that pressure between the surface of the lung and adjacent structures would be uniform throughout the entire chest. This is the situation which would obtain if the lung were inflated freely in outside air and allowed to assume a shape determined by the distensibility of the various portions of the lung. It is commonly assumed that these conditions obtain within the thorax under normal conditions, and that pressure between the visceral pleura and adjacent structures is everywhere uniform at any instant. The assumption has received some experimental support (21, 18), but the correctness of the assumption has not been universally accepted (16, 11).



If, on the other hand, the lung were prevented from assuming the freely inflated shape, the tension vector directed inward from the point of contact of the lung with the distorting body would be less than similar vectors elsewhere in the lung. Such a local diminution in tension would result in the production of inequalities of pressure between the surface of the lung and adjacent structures. In the distorted area, pressure would be higher than elsewhere because of the reduced tendency of the lung to retract from the surface of the distorting body. Pressure in the distorted area would also be elevated by the elongation of the surface of the lung, with a resulting increase in the tension forces acting parallel to the surface.

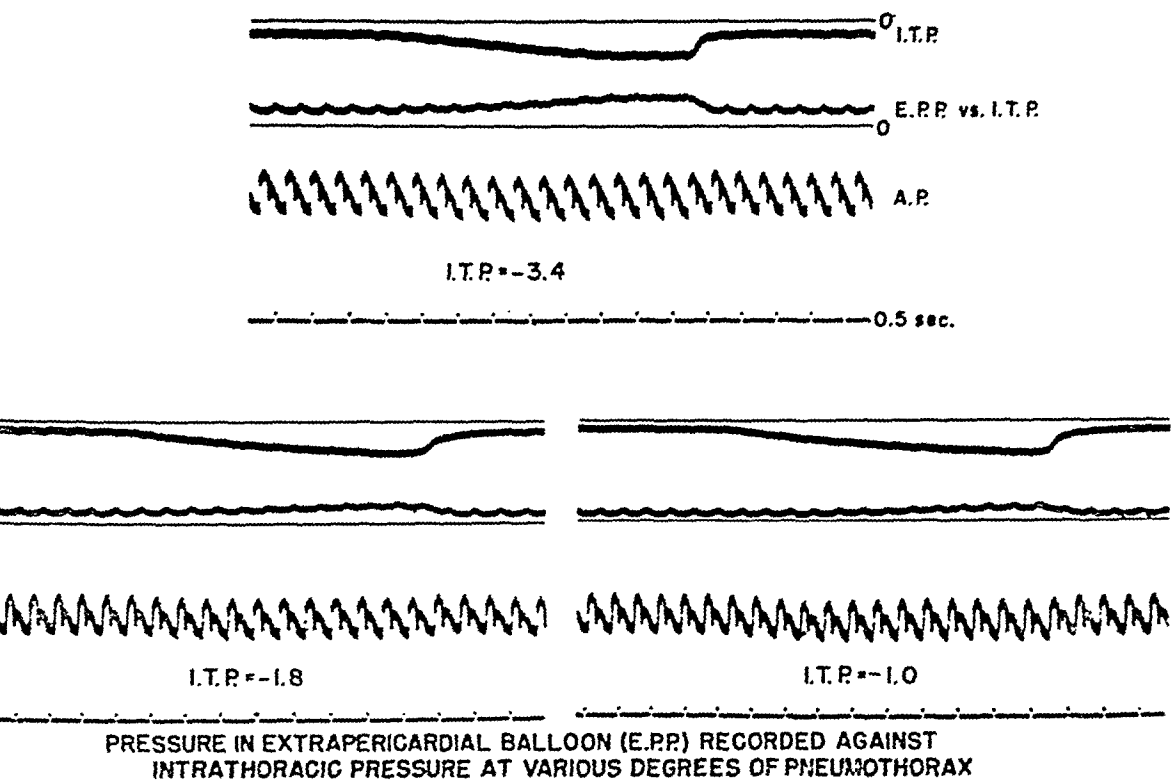


Fig. 7. Extrapericardial balloon pressure recorded differentially against pressure in a closed pneumothorax during progressive enlargement of the pneumothorax.

The present experiments have offered evidence to support this line of reasoning in the form of observations of the results of controlled distortion by a hemispherical balloon. The experiments have also shown that pressure between the pericardium and the wall of the lung is higher than pressure between the external surface of the lung and the chest wall. It has been shown in both the case of the hemispherical balloon and in the case of the extrapericardial balloon that the difference between balloon pressure and "intrathoracic" pressure depends in part upon the general state of tension throughout the lung. The parallelisms observed lead to the conclusion that, under the conditions of the experiments, the dog's heart produces deformation of the adjacent lung, raising pressure on the

external surface of the heart above the pressure existing between the lung and the wall of the thorax.

The necessity of avoiding distortion of the lung when making measurements of intrathoracic pressure has been recognized and emphasized previously (16, 18). The failure on the part of earlier investigators to detect local variations in intrathoracic pressure was apparently dependent upon the fact that the measurements were made from various sites between the lateral surfaces of the lungs and the thoracic wall. The possibility that the heart might act as a distorting body seems not to have been considered.

The term "effective filling pressure" was designed by Henderson (6) to indicate the head of pressure available for filling the ventricles during the diastolic pause. Although Wiggers (19) has defined the venous component of effective filling pressure in a specific fashion, the manner of measurement of the extracardiac component has never been adequately defined. Consequently, effective filling pressure has been measured in several different ways, some of which are open to criticism on the basis of elastic distortion of the lung (19, 4). The results presented here indicate the errors inherent in the assumption that effective filling pressure can be measured accurately as the difference between atrial pressure and the pressure existing at the lateral surface of the lung. Effective filling pressure can be measured more accurately by measuring the difference between atrial and extracardiac pressures. Inaccuracies are probably unavoidable even with this type of measurement, for there is no assurance that extracardiac pressure is uniform over the entire surface of the heart. The discrepant results of Boyd and Patras (1) and of Boyd and Brookhart (2) were apparently due to the fact that in the former instance the cardiometer shielded the ventricles from the pressure effects of the lungs, while in the latter instance the local elevation of extracardiac pressure supported atrial pressure and prevented the expected reduction.

It must be noted that these conclusions are based upon observations carried out on anesthetized dogs in the supine position. It is quite possible that alterations from this abnormal posture might influence the amount of lung distortion produced by the heart, and that in the normal, unanesthetized dog local inequalities in intrathoracic pressure might be different from those described here. The amount of distortion of the lungs produced by the heart in other species would depend entirely upon the mechanical conditions existing within the chest. Otis, Rahn and Fenn (13) have recently carried out an analysis of the physical characteristics of the human lung using an indirect approach, basing their reasoning upon an assumption which they recognize may be erroneous. They assume that, under conditions of increased intrapulmonary pressure, venous pressure and intrathoracic pressure rise by the same increment. If it can be shown that the human heart acts as the dog heart in producing distortion of the lung, then their assumption cannot be justified without further qualification.

#### SUMMARY

1. Optical pressure measurements in supine anesthetized dogs indicate that extrapericardial pressure is higher than pressure in a small closed pneumothorax;

that the difference becomes greater when the lungs are inflated either by normal respiration or by positive intrapulmonary pressure; and that the difference becomes less when the lungs are deflated by enlarging the pneumothorax.

2. Pressure within a balloon which produces distortion of the lung is shown to behave in a similar fashion.

3. It is concluded that in the dog local elevation of "intrathoracic" pressure occurs in the region of the heart because of the distortion of the lung produced by the heart.

4. The influence of local variations in "intrathoracic" pressure upon measurements of effective filling pressure is described and discussed.

5. It is concluded that effective filling pressure can be measured most accurately as the difference between intracardiac and extracardiac pressures.

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# THE RENAL TUBULAR REABSORPTION OF CHLORIDE<sup>1</sup>

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Precise clearance methods for the quantitative study of renal function have revealed three distinct types of tubular reabsorptive mechanisms. The first of these, exemplified by the mechanism for the reabsorption of urea, is dependent upon forces of diffusion. Urea is readily diffusible and passes from the glomerular filtrate into the tubular blood in consequence of a diffusion gradient established across the tubular epithelium by the reabsorption of water. Thus the quantity of urea reabsorbed per unit of time is an inverse function of the urine flow and a direct function of the plasma urea concentration. If both urine flow and plasma concentration stay constant, the quantity of urea reabsorbed per unit of time is a direct function of the rate of glomerular filtration (1) (2) (10). In contrast a second, or "active" type of tubular reabsorptive mechanism, exemplified by that for glucose (11), ascorbic acid (3) (9), amino acid (5) (6), or phosphate (7) is dependent upon forces developed within the tubular cells in consequence of their own metabolic activities. The tubules exhibit for these substances a fixed and limited reabsorptive capacity ( $T_m$ ). Presumably this limitation derives from the fact that these substances combine in their reabsorption with a fixed quantity of some component of the tubular cell. The complex thus formed then undergoes degradation, liberating the reabsorbed material into the peritubular fluid and bloodstream. When sufficient quantities of substrate are presented to the tubular cells to completely saturate the cellular component, the rate of reabsorption becomes fixed and independent of urine flow, plasma concentration, and glomerular filtration rate. A third type is exemplified by the reabsorptive mechanism for the anion, bicarbonate. This mechanism combines some of the characteristics of both the "active" and "diffusion" types of reabsorptive mechanisms. In one sense the rate of bicarbonate reabsorption is fixed and limited, in that the quantity reabsorbed per unit of time is independent of plasma concentration above the threshold for gross excretion. However the rate of reabsorption is directly related to the rate of glomerular filtration. These two features of the bicarbonate mechanism, namely, independence of rate of reabsorption and plasma concentration, and dependence of rate of reabsorption and filtration rate, combine nicely to stabilize plasma bicarbonate concentration within narrow limits that are independent of functional changes in the rate of glomerular filtration.

Bicarbonate and chloride are closely related in the electrolyte economy of the

<sup>1</sup> This study was aided by a grant from the John and Mary R. Markle Foundation of New York.

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body. Together they form the major acid complement of body base. Variations in the plasma concentration of the one are accompanied by opposite and reciprocal variations in the plasma concentration of the other, so that total plasma anion concentration tends to remain constant. Because of these close functional relations it might be assumed that both ions are reabsorbed by mechanisms of similar properties. This assumption is borne out by experiment. During the course of a study performed for another purpose it became evident that functional increases in the rate of glomerular filtration are accompanied by nearly proportional increases in the capacity of the renal tubules to reabsorb chloride. The data upon which this statement is based are presented below.

PROCEDURE. Experimental procedures and analytical methods were similar to those reported elsewhere (8) (4) (7). Because of other observations being

TABLE 1

*An experiment on a dog illustrating the relationship between glomerular filtration rate and the tubular reabsorption of chloride. Filtration rate was increased by the intravenous infusion of alanine*

TOTAL CONCURRENT TIME	URINE FLOW	ARTERIAL PLASMA			GLOMERU- LAR FIL- TRATION RATE	CHLORIDE			
		Creatinine	Chloride	Amino-N		Filtered	Excreted	Reabsorbed	
min.	ml./min.	mgm. %	mM./L	mgm. %	ml./min.	mM./min.	mM./min.	mM./min.	mM. per 100 ml. filtrate
95-105	6.8	23.2	126	4.92	63.6	8.01	0.48	7.53	11.8
105-115	6.4	22.9	126	4.65	63.4	7.98	0.91	7.07	11.2
130-140	7.4	23.3	127	7.49	63.5	8.06	0.93	7.13	11.2
140-150	7.6	23.0	127	7.95	65.8	8.35	0.99	7.36	11.2
165-175	9.4	23.3	127	11.9	77.7	9.76	1.31	8.45	10.9
175-185	9.7	24.0	127	13.0	78.8	10.1	1.28	8.81	11.2
200-210	10.8	24.1	127	17.4	87.0	11.1	1.66	9.39	10.8
210-220	11.0	23.8	128	18.7	92.0	11.8	1.66	10.2	11.1

made simultaneously, the dogs were in a state of acidosis, having received 500-600 ml. of 3 per cent ammonium chloride during the two days prior to each experiment. Filtration rate was altered physiologically by the intravenous administration of amino acids.

RESULTS. Data obtained in a representative experiment are presented in table 1. This experiment illustrates the response of the chloride reabsorptive mechanism to an increase in the rate of glomerular filtration. Plasma amino acid was elevated stepwise by the intravenous administration of alanine. The result was a characteristic increase in filtration rate from an initial value of 63.5 ml. per minute to a figure of 92 ml. per minute in the last period. Notice that plasma chloride concentration, although slightly above normal because of acidosis and the infusion of 0.85 per cent sodium chloride, stayed remarkably constant throughout the experimental periods. Therefore any change in the capacity of the tubules to reabsorb chloride was conditioned not by any change in

plasma chloride concentration, but rather by the change in the rate of glomerular filtration. Reference to the right hand side of the chart illustrates the quantitative nature of this change in chloride reabsorptive capacity. It is evident in this case that a functional increase in glomerular filtration rate was accompanied by an almost proportional increase in the capacity of the renal tubules to reabsorb chloride when that capacity is expressed in millimols per minute. Accordingly when that capacity is expressed in millimols reabsorbed from each 100 ml. of glomerular filtrate, the quantity remained essentially unchanged.

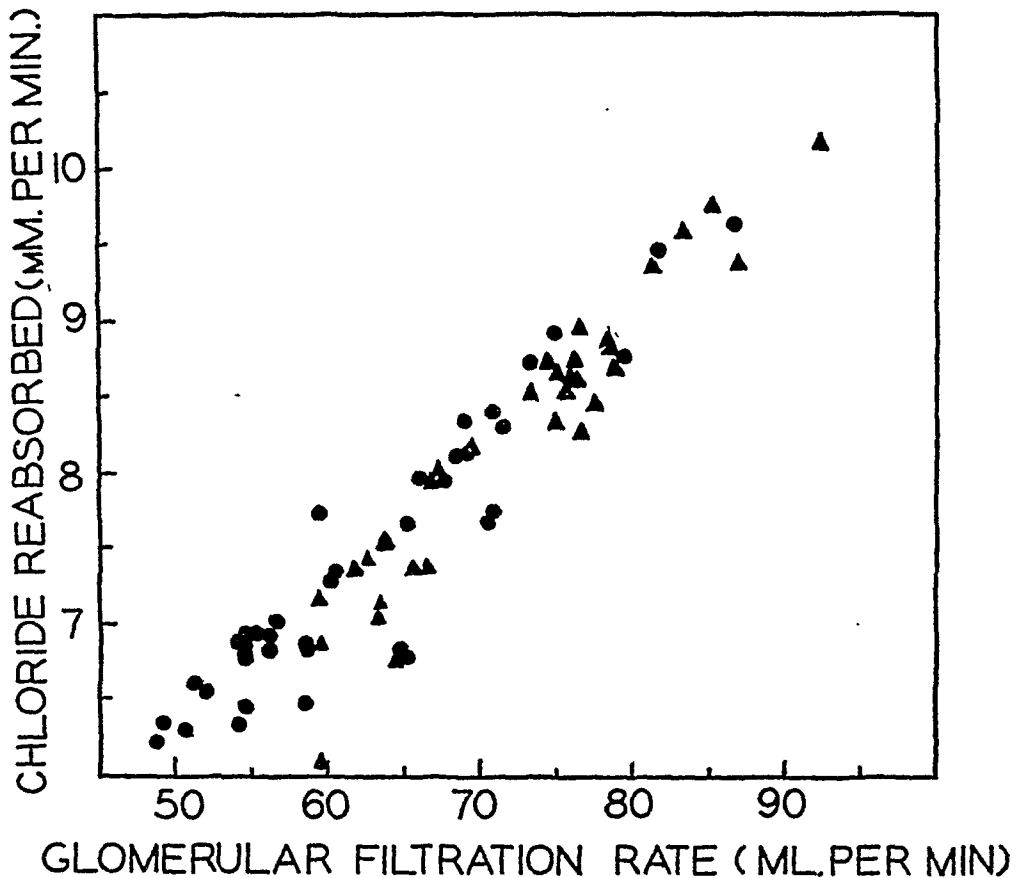


Fig. 1. The relationship between chloride reabsorption in millimols per minute and glomerular filtration rate in milliliters per minute.

This relation between chloride reabsorptive capacity and glomerular filtration rate is further illustrated by the plot of figure 1. This figure summarizes the data of sixteen experiments on two dogs. Each point represents a single clearance period. The chloride reabsorptive capacity is here expressed in millimols reabsorbed per minute and is plotted against glomerular filtration rate which varied in these experiments from a low of 48.8 ml. per minute to a high of 93.4 ml. per minute. These data were all obtained during experiments in which plasma chloride remained essentially constant at levels sufficiently above normal to effect gross excretion of chloride. There is evident in this plot the same linear relationship between chloride reabsorptive capacity and glomerular filtration rate as was seen in the data of table 1. This proportion is almost a direct one in the massed

data, and represents a definite and reproducible characteristic of the chloride reabsorptive mechanism.

**DISCUSSION.** The mechanism for the reabsorption of chloride plays a significant rôle in the stabilization of plasma chloride concentration. The level of plasma chloride in the normal individual is maintained within a range of 100 and 110 millimols per liter; at somewhat higher levels in acidosis. In these experiments plasma chloride was sufficiently increased by the infusion of sodium chloride to exceed the renal threshold and cause the frank excretion of chloride. Due to the acidosis the renal threshold was no doubt somewhat elevated as compared to the normal, for the renal tubules were capable of reabsorbing on an average 11.5 millimols of chloride from each 100 ml. of glomerular filtrate. Because of the reabsorption of this quantity of chloride, the plasma concentration would tend to stabilize at 11.5 millimols per 100 ml., or as is usually expressed, at 115 millimols per liter.

The tubular mechanisms for the reabsorption of bicarbonate and chloride bear close resemblance in that both are direct functions of glomerular filtration rate. Such a resemblance is reasonable because of the close association of these two ions in the bodily economy. These experiments represent by no means a complete elucidation of the complex mechanism for the tubular reabsorption of chloride, but are presented here for the purpose of pointing out the relations between the chloride reabsorptive capacity and glomerular filtration rate, and to classify this process in the same general functional category with the reabsorptive mechanism for bicarbonate.

#### SUMMARY

In a series of sixteen experiments on two dogs the capacity of the renal tubules to reabsorb chloride has been studied in relation to changes in the rate of glomerular filtration, physiologically produced by the intravenous administration of amino acid. It has been found that functional changes in the rate of glomerular filtration are correlated with direct and nearly proportional changes in the capacity of the renal tubules to reabsorb chloride. Thus the renal excretory threshold for chloride is nearly independent of the rate of glomerular filtration and in this respect the mechanism for the reabsorption of chloride resembles that for bicarbonate.

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# THE RELATION OF SERUM POTASSIUM TO SHOCK IN DOGS SUBJECTED TO MUSCLE TRAUMA<sup>1</sup>

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Zwemer and Scudder (1) and Scudder (2) have reported that the potassium concentration of the blood increases during shock and they have observed terminal values comparable to those seen when death occurs following the injection of potassium into normal animals. The purpose of the present study has been to determine the relation between the rise in serum potassium and the development of the clinical signs of shock.

**PROCEDURE.** The data on serum potassium changes were obtained from a series of dogs in which Gregersen and Root (3) produced shock by muscle trauma. Their report should be consulted for a detailed description of the experimental procedures. Of the 40 dogs on which serial analyses of serum potassium were done, 26 died in shock and 14 survived. In addition terminal samples were obtained from a few animals not included in this group. Serum from arterial blood samples was dry ashed and then analyzed for potassium according to the method of Shohl and Bennett (4).

**RESULTS.** The changes in serum potassium shown in figures 1 and 2 were plotted as deviations from the pre-anesthetic control values. The control values ranged from 3.7 to 5.9, the average being 4.6 m.eq. per liter. It may be seen from figure 1 that the elevation of serum potassium was never greater than 1.5 m.eq. per liter until approximately  $1\frac{1}{2}$  hours before death. During the hour preceding death, however, the serum potassium invariably increased. In terminal blood samples taken from 37 animals that died in shock, the serum potassium ranged between 6.3 and 13.7 m.eq. per liter, the average being 9.1. Correlation of these data with clinical observations on the dogs reveals that the signs of shock appear long before the terminal rise in serum potassium.

The data on 14 animals that survived trauma demonstrate clearly that the elevation of serum potassium is not related to the onset of the clinical signs and symptoms of shock but is associated with certain changes that set in shortly before death. Of these animals all but one had typical signs of shock and a 30 to 40 per cent reduction of blood volume. They presented a picture, which as far as one could evaluate clinically, was identical with that observed in the dogs that died. Nevertheless, it may be seen from figure 2 that the rise in serum potassium never exceeded 1.6 m.eq. per liter.

The rise in serum potassium in the terminal stages of shock has also been found

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by several other investigators (5, 6, 7, 8). This terminal rise of serum potassium is not peculiar to the shock resulting from muscle trauma, but occurs also in intestinal obstruction (9) and in hemorrhage (10, 11).

It is important to note that the serum potassium values obtained immediately after trauma may have been influenced somewhat by the use of ether anesthesia.

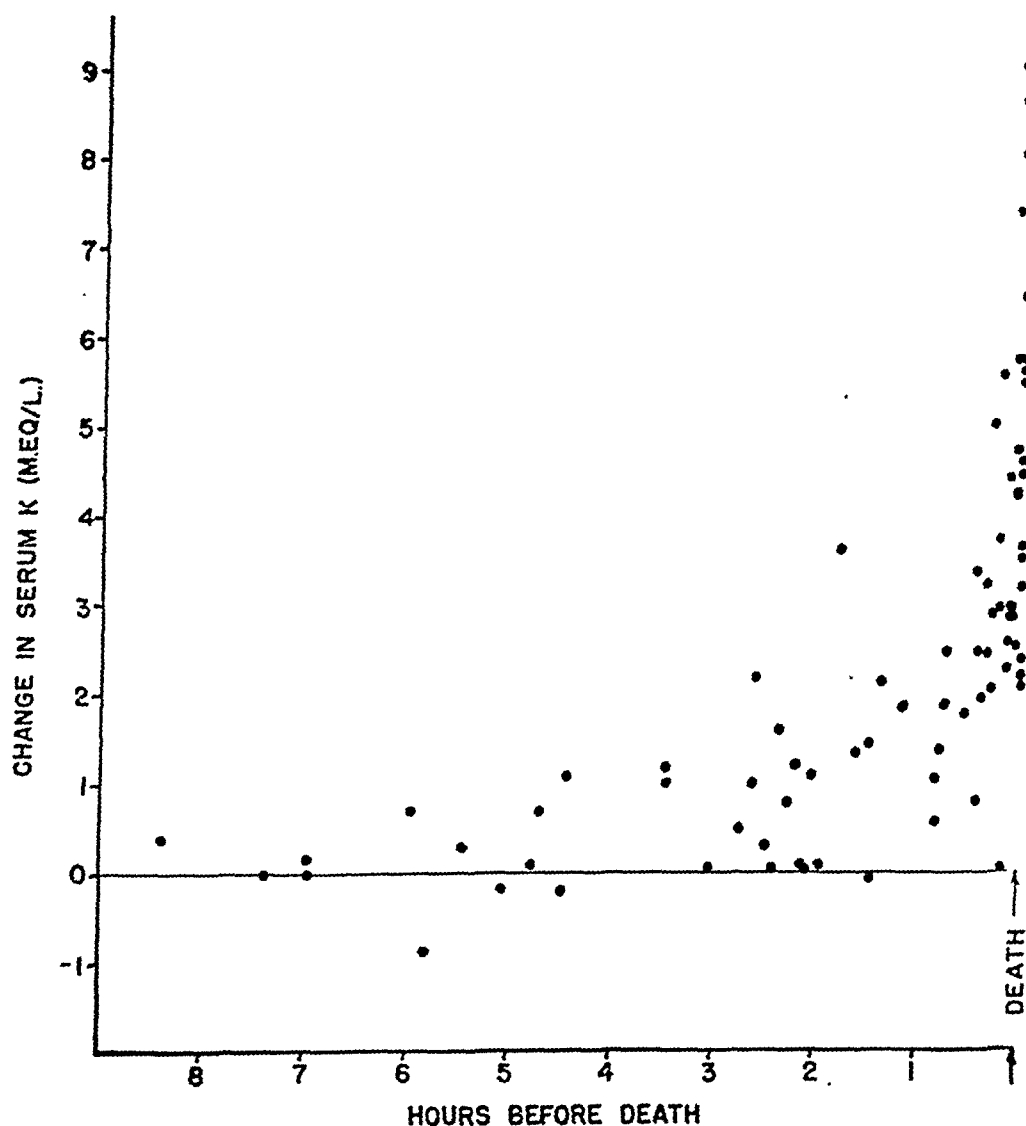


Fig. 1. Change in serum potassium following muscle trauma in 26 dogs. The effect of muscle trauma on the serum K of 26 dogs that died in shock. The changes are shown as deviations from the control value, plotted with reference to the time of death. Note the terminal rise in serum K and the absence of marked changes prior to that time.

Robbins and Pratt (12) found that in normal dogs ether lowered the serum potassium 1 to 2 m.eq. per liter and showed that this reduction might last for as long as 30 minutes after the anesthesia was discontinued. The effect of trauma uncomplicated by ether anesthesia was shown by several experiments on chronic spinal animals prepared in this laboratory by Dr. W. S. Root. Although in these

dogs the potassium levels immediately after muscle injury were 1 to 2 m.eq. higher, subsequent values were identical with those found in animals anesthetized during the period of trauma.

Gregersen and Root (3) have called attention to the fact that in their animals one of the characteristic signs of impending death was a decrease in the heart rate. The occurrence of bradycardia at a time when the serum potassium is maximal suggests a causal relation between the increased serum potassium and the terminal cardiac slowing. Winkler, Hoff and Smith (13) demonstrated that during the injection of potassium into the normal dog, intraventricular block and disappearance of the P wave occurred at serum potassium levels of 9 to 11 m.eq. per liter. Cardiac arrest was observed at serum potassium concentrations of 14 to 16 m.eq. per liter. Scudder, Smith and Drew (14) reached similar conclu-

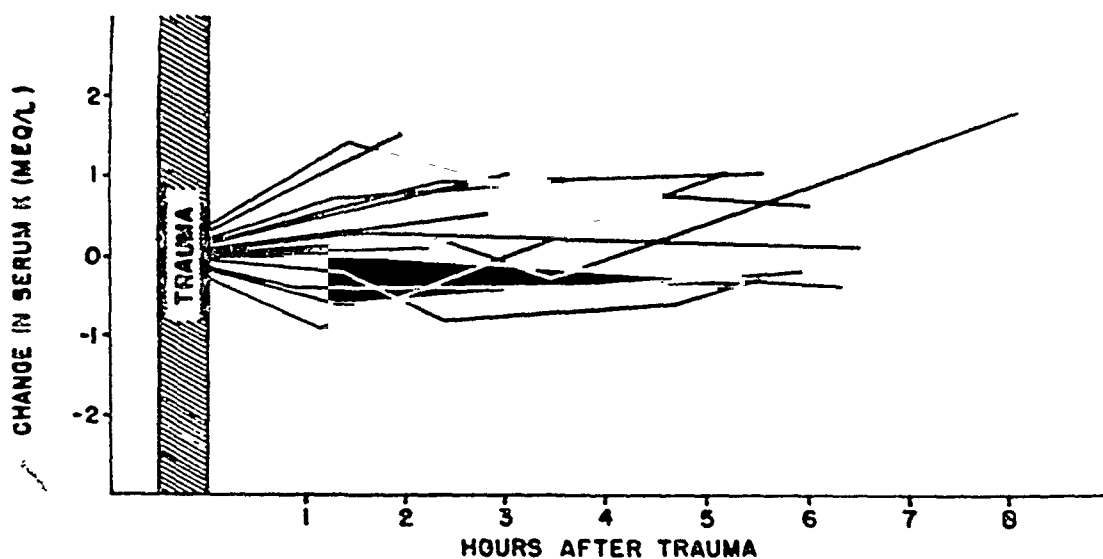


Fig. 2. Change in serum K in 14 dogs that survived muscle trauma. The effect of muscle trauma on the serum potassium of 14 dogs that survived the injury. All changes are related to time of trauma and plotted as deviations from the control value. Note the absence of any marked elevation of serum K. All but one of the dogs had fully developed signs of shock.

sions. The highest serum potassium value found in the present study was 13.7, and only 54 per cent of the animals had terminal values greater than 9 m.eq. per liter. The terminal cardiac slowing can be attributed to the increased concentration of potassium only if one assumes that the threshold for potassium effects is reduced during shock or that already initiated vagal activity may be enhanced by small elevations in serum potassium. That the latter may be the case is suggested by the observations of Hoff, Humm and Winkler (23) who showed that the effectiveness of vagal stimulation is increased by serum potassium levels ranging from 6.5 to 9.5 m.eq. per liter.

Horton (15) has shown that injury to muscle cells causes a release of intracellular potassium. Although the effects of ether partially mask the increase in serum potassium immediately after trauma, the short period of anesthesia cannot ac-

count for the continued absence of hyperpotassemia in the animals that survived. Furthermore, excretion by the kidney cannot be a factor in keeping the potassium level down because little or no urine is formed during the acute stages of shock. There appear to be two possible explanations for the absence of hyperpotassemia in non-fatal shock: *a*, that muscle potassium is either not released by the trauma, or if released does not enter immediately into the circulation but remains in the traumatized area; *b*, that potassium enters the circulation from the injured area at a slow rate and is taken up by uninjured tissue cells probably in the liver and muscle.

With respect to release of potassium from the traumatized muscle, several investigators have made tissue analyses from the area of injury. In the dog in shock produced by the Blalock press technique, Ricca et al. (16) found no change in potassium content of the muscle of the injured leg when compared with muscle tissue from normal animals, and Brues and his collaborators (7) observed no loss of potassium except in the damaged muscle directly under the press. Manery and Solandt (6) in shock produced by muscle trauma found potassium loss in the traumatized muscles of 2 of the 4 animals studied, but again the potassium loss was large only in one area where broken muscle bundles were observed. That some potassium loss does occur from the muscles is suggested by the observation that free fluid in the injured leg has a higher potassium content than samples of circulating plasma taken at the same time (6, 16). While these findings indicate a loss of potassium from the injured muscle such loss is apparently appreciable and consistent only where the gross damage is visibly evident.

There is also evidence that the potassium released from muscle cells by trauma may not leave the injured leg. Manery and Solandt (6) found that the femoral arterio-venous potassium differences were "surprisingly small". Holmes and Painter (17) compared the total potassium content of the traumatized hind leg with that of the opposite uninjured leg. By this method of comparison, these authors found in 3 animals no loss of potassium from the injured leg and in the other 3 animals a small loss, the maximum being 7 m.eq. which is not sufficient to account for the terminal rise of potassium seen in many instances. When the traumatized animal recovers from shock he excretes excessive amounts of potassium (24) which suggests that as the circulation improves during recovery, potassium released from the tissues by injury then enters the blood stream and is excreted.

The concept that uninjured cells may take up any potassium released from the injured area is supported by two pieces of evidence: *a*, injected potassium can be taken up by the muscle cells of the normal animal (25, 26); *b*, an increase in the potassium content of liver and non-traumatized muscle occurs in shock produced by the Blalock press technique (16). Apparently the absence of hyperpotassemia in the early stages of fatal shock and in the animals which survive muscle trauma can best be explained by assuming that very little potassium is released from the injured area during the acute phases of shock. In

addition there is evidence that such potassium as may be released into the circulation can be taken up by the non-traumatized tissue cells.

The cause of the terminal rise in serum potassium is not altogether clear. However, there are several factors which are known to produce an increase in serum potassium in the experimental animal and which may be operating in shock. It has been shown for example that a rise in serum potassium occurs in asphyxia (8, 18, 19) and after intermittent or partial reduction of blood flow to the tissues (20, 21). These observations are of particular interest in view of the fact that in experimental traumatic shock identical with that studied in the present series of experiments, Root, Walcott and Gregersen (22) found large and progressive reductions in cardiac output, in oxygen consumption and in the oxygen content of the mixed venous blood. The terminal rise in serum potassium may, therefore, be caused by loss of potassium from non-traumatized cells which have been deprived of their circulation as well as by the inability of these cells to continue to take up any potassium released from the injured area. If this reasoning is correct one must regard the terminal rise in serum potassium as evidence of certain tissue changes that precede death.

#### CONCLUSIONS

In 40 dogs in which shock was produced by muscle trauma the appearance of the symptoms of shock was not associated with a rise in serum potassium. Hyperpotassemia is a terminal event. It is probably related to certain tissue changes that precede death.

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# CIRCULATORY AND FLUID COMPARTMENT PHYSIOLOGY IN THE NORMAL MONKEY WITH ESPECIAL REFERENCE TO SEASONAL VARIATIONS<sup>1</sup>

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Although the volumes of the total body water, total blood, plasma and "extracellular" fluid have been measured both in man (1, 2, 3) and in a number of animals (3, 4, 5) used for experimental purposes, such data are not available for as commonly employed an animal as the monkey. The results of measurements of this sort in the normal monkey form the basis of this report.

**MATERIALS AND METHODS.** Twenty-three *Macacus mulata* monkeys, selected at random from a normal colony and ranging in weight from 2.45 to 4.40 kgm., were used. Plasma volumes were determined by a modification of the T-1824 dye dilution method described by Gibson and Evans (6). The total blood volume was calculated from the plasma volume and the average of two hematocrits. Hematocrits (control and final samples) were measured in Wintrobe tubes following centrifugation of the heparinized blood for one-half hour at 3000 r.p.m. The erythrocyte mass was calculated by subtracting the plasma volume from the total blood volume. "Extracellular" fluid volumes were measured by a modification of the NaSCN dilution method of Crandall and Anderson (3). Circulation times were measured over the femoral vein to tongue route by the fluorescein method of Fishback (7). Whole blood and plasma specific gravity were measured by the CuSO<sub>4</sub> method of Phillips, Van Slyke et al. (8) and the plasma protein concentration was derived from the plasma specific gravity. Total circulating protein was calculated from the plasma protein concentration and the plasma volume. Arterio-venous oxygen differences were determined by the micro-gasometric method of Roughton and Scholander (9). Plasma bilirubin was measured in some of the animals by the photoelectric colorimeter method of Malloy and Evelyn (10).

The volume determinations were carried out in the following manner. The femoral artery and vein of the monkey were exposed under local procaine infiltration (2 ml. of a 2 per cent solution). Three milliliters of blood were withdrawn without stasis from the vein into a 5 ml. heparinized syringe. One milliliter of this sample was used for a hematocrit determination. The other 2 milliliters were delivered into a heparinized (1 drop of liquid heparin) tube, cen-

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trifuged, and the plasma separated for use as the control (dye and NaSCN-free) reading on the Coleman Junior photoelectric spectrophotometer. The needle used to draw the control sample was left in the vein and 0.25 ml. of the dye T-1824 (0.5 per cent solution) was injected through it from a calibrated syringe. After washing out any residual dye by repeated rinsing with blood, the dye syringe was detached from the needle and 0.5 ml. of a 5 per cent NaSCN solution was injected from a second calibrated syringe. After blood-rinsing the NaSCN syringe, the needle was withdrawn from the vein and light stasis applied until bleeding ceased (usually less than 1 min.).

One-half hour elapsed before the first dyed sample was withdrawn. Thereafter, four venous blood samples were taken at 10 minute intervals. Plasma volumes were calculated after extrapolating the resulting time-concentration curve to zero time. Except for the first (control) and the last (which were 3 ml.) the samples taken were 2 ml. A second hematocrit was determined on 1 ml. of the final blood sample and the average of the two measurements was taken to be the true large vein hematocrit.

Following the withdrawal of the last dye-NaSCN sample, 1 ml. of femoral venous and 1 ml. of femoral arterial blood were airlessly withdrawn as near simultaneously as possible and the oxygen content determined by the Roughton-Scholander method.

**RESULTS.** The fluid compartment volumes of the monkey show a seasonal (temperature) variation similar to that described in the human by Conley and Nickerson (11). Since our animals were housed in a room whose temperature varied with the season, determinations made during the winter and summer months will be considered separately.

*Plasma volume.* In the 13 animals studied during the *winter* months (November to March), total plasma volumes ranged, in a normally distributed manner, from 105.1 to 178.4 ml. with a mean value of  $144.7 \pm 19.6$  ml. When plasma volume is related to the body weight, the range is 30.9 to 60.4 ml. per kilogram with a mean value of  $43.3 \pm 7.3$  ml. per kilogram (table 1). In contrast, the 10 monkeys measured during the *summer* show total plasma volumes with a mean value of  $187.5 \pm 70.0$  ml. (range 124.8 to 334.1 ml.) while the volume per kilogram has a mean of  $46.7 \pm 10.4$  ml./kgm. (range 33.5 to 63.4 ml./kgm.). The high standard deviation noted in animals studied during warm weather indicates a greater variability from animal to animal during this season.

While the seasonal differences in plasma volume in the monkey do not show high significance when statistically compared they are consistent with previous observations made on man (11).

*Blood volume and erythrocyte mass.* Since the mean hematocrit values of normal monkeys are practically identical in winter and summer (40.9 per cent in winter, 39.8 per cent in summer (table 1)), it follows that the total blood volume and total erythrocyte mass will likewise show only minor seasonal variations. Total blood volumes measured during the cold period averaged  $308.2 \pm 115.0$  ml. Similar differences were noted in the blood volume expressed in terms of body weight. Likewise, the total erythrocyte mass varied from a mean of  $102.1 \pm 16.0$  ml. in winter to  $120.6 \pm 49.8$  ml. in summer.

"Extracellular" fluid volume. The only values obtained which reveal statistically significant seasonal variations in the monkeys in this series are the

TABLE 1  
*Physiological values in 23 normal monkeys (seasonal comparison)*

SUMMER ANIMALS	PV	BV	ECF	HCT.	PL. PROT.	TCPR	A-V O <sub>2</sub>	C.T.
	cc/kgm.	cc/kgm.	cc/kgm.	per cent	grams per cent	grams	vol. %	sec.
1	49	86		42.0				
2	59	109		46.0	7.18	11.6		
3	62	105		41.0	6.78	22.6		
4	46	74		38.5	6.83	12.7		
5	39	70	290	43.4				
6	37	63	180	40.8	8.58	10.7		
7	34	58	270	42.3	8.23	10.5		
8	38	52	290	36.6	6.85	9.2		
9	63	90	250	29.8	8.58	23.9		
10	41	67	210	37.6	6.85	10.3		
Average...	47	77	250	39.8	7.48	13.9		
Std. Dev...	10.4	18.4	40.7	4.3	0.77	5.5		
WINTER ANIMALS								
11	43	81	240	46.2	7.18	10.2	6.3	7.5
12	31	59	180	45.4	6.85	8.2	4.8	12.0
13	41	71	180	43.1	6.46	8.3	11.8	15.0
14	49	75	180	34.1	7.37	12.0	7.5	9.8
15	45	71	170	35.8	6.28	10.1	6.1	8.5
16	31	63	170	49.0	6.82	7.2	6.4	8.8
17	45	77	220	41.1	6.28	9.1	5.2	8.6
18	40	69	160	41.3	6.46	10.1	6.6	8.4
19	42	73	160	42.9	7.18	9.2	9.5	8.8
20	43	79	180	45.0	6.10	9.9	8.9	10.0
21	60	85		29.0	6.28	11.2	5.8	7.0
22	41	73		41.9	6.28	9.2	3.6	
23	48	76		36.3				
Average...	43	73	184	40.9	6.63	9.6	6.9	9.5
Std. Dev...	7.3	6.7	25.2	5.4	0.40	1.2	2.2	2.2

PV = plasma volume

BV = blood volume

ECF = extracellular fluid volume

Hct. = hematocrit

TCPr = total circulating protein

A-V O<sub>2</sub> = femoral arterial-femoral venous blood O<sub>2</sub> content

C.T. = circulation time (femoral vein to tongue)

measurements of "extracellular" (available) fluid volumes. Both the total and "extracellular" fluid volume per kilogram are significantly higher during warm periods than during cold periods. The average total "extracellular" fluid volume of the monkey in winter is  $634.2 \pm 82.3$  ml., while in summer the value is 878.5



$\pm 166.2$  ml. Winter-studied monkeys have an "extracellular" fluid volume of 18.6 per cent of the body weight which increases to 24.7 per cent in the summer group (table 1).

*Plasma proteins.* Neither the protein concentration of the plasma nor the total circulating protein showed any constant seasonal variation (table 1). The method used for the determination of the protein concentration ( $\text{CuSO}_4$ ) is not one of high accuracy (12) and further study using other methods might reveal some consistent minor variations. The average plasma protein concentration of all 23 monkeys was  $6.97 \pm 0.55$  grams per cent while the total circulating protein averaged  $11.3 \pm 3.5$  grams.

*Arterio-venous oxygen differences.* Since summer and winter hematocrit values in the normal monkey are practically identical, arterial and venous blood oxygen content measurements were abandoned as it seemed unlikely that they would show any seasonal changes. Such measurements were made, however, on 12 of the 13 winter-studied animals which had an average A-V  $\text{O}_2$  difference of  $6.9 \pm 2.2$  volumes per cent (table 1).

*Circulation time.* Employing a modification of Fishback's (7) fluorescein method, circulation times were measured on 11 of the 13 winter-studied animals (table 1). One-half milliliter of a 5 per cent solution of sodium fluorescein was injected into the exposed femoral vein and the time required for the fluorescein to reach the tip of the tongue was designated as the circulation time. Observations were carried out in a dark room under ultra-violet illumination. Repeated experiments with circulation times from the femoral vein to various points on the head (tongue, conjunctival mucosa, eyebrows, lips) revealed that more constant values could be obtained most readily by observing the tip of the tongue. The range in circulation times for this route was 7.0 to 15.0 seconds in the normal animal with a mean value of  $9.5 \pm 2.1$  seconds.

*Discussion.* The average values obtained for blood, plasma, and "extracellular" fluid volumes in the monkey, when expressed as per cent of body weight, are quite similar to values previously described in the human. Thus, while the average normal plasma volume in man has been reported to be  $45.0 \pm 4.0$  ml./kgm. (2), the average plasma volume of 23 monkeys (both winter and summer) was  $44.9 \pm 7.0$  ml./kgm. The average normal human whole blood volume is  $85.0 \pm 8.9$  ml./kgm. (2) while the monkey has an average whole blood volume of  $75.1 \pm 12.0$  ml./kgm. Likewise, NaSCN ("extracellular") volumes in man have been reported to average 24.3 per cent of the body weight (3); the average for the monkeys in this series is 20.9 per cent. It is not surprising, therefore, to find that seasonal variations in the size of the fluid compartments occur in the monkey as well as in man. It may well be that the larger changes found in the "extracellular" fluid volume in monkeys than occur in the human are related to the fact that the monkey, being a fur-bearing animal, has a less efficient sweating mechanism.

There is an indication from the comparison of the winter and summer "available" fluid volumes in the monkey, that the extracellular compartment considered as a whole is considerably more labile than the plasma volume alone.

The revelation of this lability lies in the fact that only the NaSCN volume shows a statistically significant alteration with the season. Whether this is a real change in the size of the "extracellular" compartment cannot be definitely ascertained from these data since a small change in the permeability of the tissue cells to the SCN ion during the warmer period would likewise result in an *apparent* increase in "extracellular" volume.

#### SUMMARY

1. The plasma, blood and "extracellular" fluid volumes, hematocrit, circulation time, plasma protein concentration, total circulating protein and arterio-venous oxygen differences were determined in a group of 23 normal monkeys (*Macacus mulata*).

2. There appears to be a seasonal variation in fluid volumes in the monkey similar to alterations previously reported in man subjected to prolonged warm and cold periods.

3. The only compartment showing a statistically significant seasonal variation in the monkey is the "extracellular" fluid volume.

4. The similarity between the values reported here and those obtained in human patients by other workers is indicated.

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# SECRETORY FUNCTION OF SYMPATHETIC NEURONES AND SYMPATHIN FORMATION IN EFFECTOR CELLS<sup>1</sup>

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The existence of a secretory function of postganglionic adrenergic neurones, with their ganglion cells serving as indispensable "trophic" centers, has been postulated by some investigators on the grounds of morphological findings, such as the presence in sympathetic ganglia of chromaffin cells similar to those of the adrenal medulla (1), and histological signs of a secretory activity of sympathetic ganglion cells (2, 3). Young (4) maintains that the inside of nerve axons consists of a viscous fluid which flows down the fibre but will stagnate and be ultimately removed if the head of pressure has been cut off through severance of the nerve fibre.

The identity with epinephrine of at least part of the sympathomimetic substances present within the adrenergic neurones was demonstrated by Cannon and Lissák (5) and by Lissák (6) through biological tests carried out with extracts made of such neurones (ganglia and postganglionic fibres) with Loewi's (7) dialyzation method. Shaw's (8) colorimetric reaction for epinephrine was likewise found by Cannon and Lissák (5) to be positive in extracts of the mesenteric nerves but no quantitative chemical determinations were carried out by these authors.

Evidence for the release of epinephrine-like chemical neuro-transmitters from stimulated sympathetic neurones has been presented by O. Loewi (7), Cannon and Rosenblueth (9) and Gaddum and Kwiatkowski (10). Büllbring (1) demonstrated the appearance of epinephrine in the perfusate of sympathetic ganglia during and immediately following electrical stimulation of their preganglionic fibres.

During the past 7 years one of us (R.) has made extensive use of Shaw's colorimetric method for the quantitative assay in blood and tissues of epinephrine and related catechol compounds by applying a slight modification which was based, in part, on suggestions made by Bullen and Bloor (11). The results were in reasonable agreement with those obtained with quantitative biological tests (see table 1) and revealed physiological and pathological changes attributable to adreno-sympathetic functional alterations (12).

In an effort to obtain quantitative chemical evidence for the supposed process of sympathetic neurosecretion, the modified method of Shaw was applied to sympathetic ganglia and fibres and to effector cell tissue (heart muscle) under standard conditions as well as following electrical stimulation and resection of ganglia.

<sup>1</sup> This study was aided by a grant from the John and Mary R. Markle Foundation.

**METHODS.** Dogs were anesthetized with nembutal and operated on or they were killed without previous experimentation through intravenous injection of 20 per cent magnesium sulphate.

Operations consisted of sterile excision of the coeliac ganglion or attachment of shielded Harvard electrodes to the coeliac ganglion and adjacent postganglionic fibres or excision of the coeliac ganglion followed by attachment of the electrodes to the severed postganglionic fibres of the mesenteric plexus.

The faradic stimulations were carried out with a Harvard inductorium, connected to alternating current by means of a transformer and a resistor.

For chemical analysis the coeliac ganglion and the postganglionic fibres of the mesenteric plexus were removed separately, stripped of fat, connective tissue and vascular branches, dried superficially with filter paper, weighed, cut finely in 10 per cent trichloroacetic acid, ground with Ottawa sand, filtered and worked up as described in a previous publication (13). The thoracic sympathetic trunk was

TABLE 1

*Comparison of quantitative biological and colorimetric determination of sympathomimetic amines in various tissues*

TYPE OF TISSUE	BIOLOGICAL RESULTS ( $\gamma$ /GM.)	AUTHOR	COLORIMETRIC RESULTS ( $\gamma$ /GM.)*	AUTHOR
Heart muscle (cat).....	0.5-0.8	Cannon & Lissák (5)	0.9-1.8	Raab
Spleen.....	2-10	von Euler (14)	1-5	Raab
Adrenal glands.....	150 -1320	Shaw (8)	115 -502	Raab
Sympathetic neurones .....	4 -4.5	Lissák (6)	2.1-4.7	Raab
Vagus (cervical)....	0-1	Lissák (6)	0.06-0.2	Raab
Sciatic nerve.....	0.6-1	Lissák (6)	0.09-0.4	Raab
Phrenic nerve.....	0	Lissák (6)	0	Raab

\* Color units expressed in epinephrine equivalents (see p. 461).

laid on filter paper and the ganglia were cut off and discarded while the fibres were used for analysis.

The colorimetric readings are expressed in color units, one unit corresponding in color intensity to 0.001 gamma of epinephrine. The qualitative composition of the values found, regarding the relative proportion of epinephrine proper on one hand, and of related epinephrine-like catechol compounds on the other, can be roughly estimated by the d.s.r. (denominator of specific ratio), indicating the relation between the final color intensities of the alkali- and acid-treated specimens (13). A d.s.r. of 2.00 or more indicates the presence of practically pure epinephrine, while lower figures, down to 1.00 or below, are suggestive of an increasing admixture of other epinephrine-like compounds. Ascorbic acid participates in the colorimetric reaction involved in the method; however, its color intensity is so weak (1/320 of that of epinephrine) that it does not influence the readings to a great extent.

In a few experiments coeliac ganglia and mesenteric fibres were subjected to the extraction and dialyzation method of O. Loewi (7), as modified by Cannon and

Lissák (5), and the dialysates were tested against known amounts of epinephrine on the blood pressure of atropinized, cocainized cats.

For determination of sympathetic discharges into the heart muscle, one or both stellate ganglia of cats under nembutal anesthesia, with the adrenal glands tied, were exposed and severed from the thoracic sympathetic chain. Shielded electrodes were attached to the ganglia or, as in another group of experiments, to the postganglionic sympathetic fibres after the latter had been severed from the stellate ganglia and the ganglia had been excised. The hearts were worked up chemically immediately after cessation of electrical stimulation of the nerves.

Calculations of Fisher's *t*-test for significance of the differences found were kindly carried out by Dr. William v. B. Robertson.

TABLE 2

*Epinephrine and epinephrine-like chromogenic material in sympathetic neurones of dogs (controls)*

DOG NO.	THORACIC TRUNK		COELIAC GANGLION		POSTGANGLIONIC MESENTERIC FIBRES	
	AC*	d.s.r.†	AC*	d.s.r.†	AC*	d.s.r.†
1	1288	1.77	5584	1.10	2636	1.24
2	3361	3.20	7888	1.13	2511	2.15
3	540	1.43	2941	1.42	2397	1.45
4	3454	1.76	5145	1.14	4309	1.57
5	696	1.60	3510	1.88	1280	1.00
6	424	2.00	8810	1.32	4613	1.86
7	539	2.40	1729	2.20	463	2.20
8	2330	2.60	3593	1.12	1905	1.60
9	451	2.60	4397	1.41	897	2.20
10	605	2.80	3888	1.38	427	1.66
Average.....	1369	2.22	4749	1.41	2144	1.69

\* The term AC (adsorbable chromogen) is being used here, as in previous publications, to indicate the total colorimetric readings, expressed in color units per gram of tissue. One color unit equals in color intensity 0.001 gamma of epinephrine.

† Denominator of specific ratio (see p. 461).

RESULTS. (a) *Standard conditions.* In 10 dogs (table 2) the thoracic sympathetic trunks (minus ganglia) contained pure or prevaillingly pure epinephrine in amounts ranging between 424 and 3454 color units per gram with an average of 1369, and an average d.s.r. of 2.22.

The chromogenic content of the coeliac ganglia ranged between 1729 and 8810 color units per gram, with an average of 4749 and an average d.s.r. of 1.41. Thus, it seemed to consist only in part of epinephrine proper.

The postganglionic mesenteric fibres were found to contain a similarly composed chromogenic material, however, in somewhat smaller amounts, ranging between 427 and 4613 color units per gram with an average of 2144 and an average d.s.r. of 1.69.

The chromogenic material in the heart muscles of 15 control cats (table 6)

ranged between 931 and 1824 color units per gram, with an average of 1449 and an average d.s.r. of 1.11. The latter indicates the presence of a relatively high proportion of epinephrine-like substances other than epinephrine proper.

(b) *Extirpation of the coeliac ganglion* in 8 dogs (table 3) and analysis of both the sympathetic trunks and the remaining degenerated postganglionic mesenteric fibres after intervals of 7 to 69 (average 27) days, showed no change in the thoracic trunks but an average decrease of 64 per cent of the chromogenic content of the degenerated postganglionic fibres, combined with an apparent slight shift toward epinephrine-like non-epinephrine compounds. In one dog (no. 16) the fibres had become almost entirely free of chromogenic material. The probability that the diminution of the chromogenic material in the postganglionic fibres

TABLE 3

*Epinephrine and epinephrine-like chromogenic material in sympathetic thoracic trunks and in postganglionic mesenteric fibres after excision of the coeliac ganglion (dogs)*

DOG NO.	THORACIC TRUNK		DEGANGLIONATED MESENT. FIBRES		NO. OF DAYS BETWEEN REMOVAL OF GANGLION AND DEATH OF ANIMAL
	AC*	d.s.r.†	AC*	d.s.r.†	
11	228	2.50	1607	1.33	7
12	443	?	884	2.36	14
13	574	2.80	118	0.66	14
14	1545	1.55	1613	1.25	17
15	5218	1.40	381	0.80	21
16	527	3.00	69	?	23
17	1201	2.11	561	1.69	53
18	1913	2.36	1003	1.23	69
Av.....	1456	2.25	779	1.33	27
Diff.....	+6%		-64%		

\* and † See table 2.

occurred by chance alone ( $P$  in Fisher's  $t$ -test for significance) is 0.03. Thus, the difference appears significant.

(c) *Faradic stimulation of the coeliac ganglion and adjacent postganglionic mesenteric fibres* in 8 dogs, and of the deganglionated fibres alone in 2 dogs for 12 to 300 (average 140) minutes (coil distance 7 to  $\frac{1}{4}$  cm.) was followed by an average diminution of 32 per cent of the chromogenic material in the ganglia ( $P = 0.132$ , not significant) and of 54 per cent in the postganglionic fibres ( $P = 0.05$ , significant) without any significant change of the average d.s.r. (table 4).

The lowest figures were found when stimulation had been continued over 4 to 5 hours, and after ganglionectomy.

(d) *Non-sympathetic peripheral nerves* (table 5). Very small amounts of chromogenic material, which consisted in part of epinephrine, were found in the cervical vagus and in the sciatic nerve while no chromogenic material could be detected in the phrenic, brachial and crural nerves.

TABLE 4

*Epinephrine and epinephrine-like chromogenic material in sympathetic neurones of dogs after faradic stimulation*

DOG NO.	COELIAC GANGLION		POSTGANGLIONIC MESENTERIC FIBRES		ELECTRODES ATTACHED TO :	MIN-UTES
	AC*	d.s.r.†	AC*	d.s.r.†		
19	3459	1.21	1882	?	Coeliac ganglion and mes. fibres	12
20	2512	1.21	1792	2.00	Coeliac ganglion and mes. fibres	15
21	2485	1.66	819	1.33	Coeliac ganglion and mes. fibres	30
22	2894	2.47	2200	2.00	Coeliac ganglion and mes. fibres	150
23	5366	1.54	921	1.40	Coeliac ganglion and mes. fibres	180
24	5037	1.32	298	3.00	Coeliac ganglion and mes. fibres	240
25	2369	1.04	107	2.00	Coeliac ganglion and mes. fibres	240
26	1804	1.05	493	1.41	Coeliac ganglion and mes. fibres	300
27	Ganglion removed		385	1.24	Mesent. fibres	95
28	Ganglion removed		944	1.03	Mesent. fibres	120
Av.....	3241	1.44	984	1.71		138
Diff.....	-32%		-54%			

\* and † See table 2.

TABLE 5

*Epinephrine and epinephrine-like chromogenic material in nerves other than the sympathetic (dogs)*

TYPE OF NERVE	AC*	d.s.r.†
Vagus (cervical).....	62	1.00
Vagus (cervical).....	126	1.66
Vagus (cervical).....	186	?
Vagus (cervical).....	84	1.56
Sciatic nerve.....	88	?
Sciatic nerve.....	405	0.96
Phrenic nerve.....	0	
Brachial nerve.....	0	
R. crural nerve.....	0	
L. crural nerve.....	0	

\* and † See table 2.

(e) *Biological tests on dialyzed extracts from normal and degenerated adrenergic neurones.* Extracts from normal ganglia and from postganglionic mesenteric sympathetic fibres, matched with known quantities of epinephrine regarding

their blood-pressure raising effect in the cat, elicited responses within the range of their epinephrine content, as estimated from the average chemical findings in corresponding amounts of ganglion and nerve fibre tissue which had been taken from other animals (fig. 1).

Extracts from degenerated postganglionic mesenteric sympathetic fibres, tested 36 and 60 days respectively after excision of the coeliac ganglion, produced abnormal effects on the blood pressure of the cat: an initial fall, followed by a weak or almost unnoticeable blood pressure elevation (fig. 2).

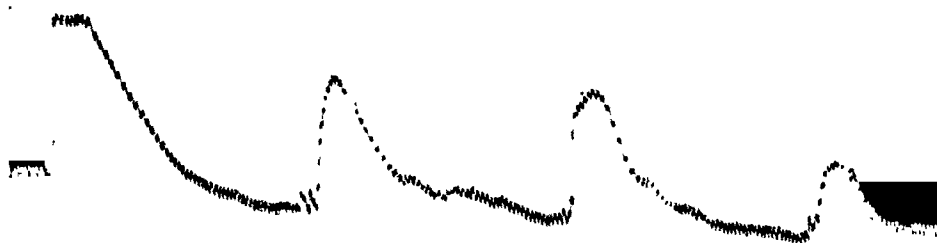


Fig. 1. Cat cocaineized (10 mgm./kgm.).

Epinephrine  
1 gamma

Postganglionic  
mesenteric fibres  
1 gamma equ.

Epinephrine  
 $\frac{1}{2}$  gamma

Coeliac  
ganglion  
 $\frac{1}{2}$  gamma equ.

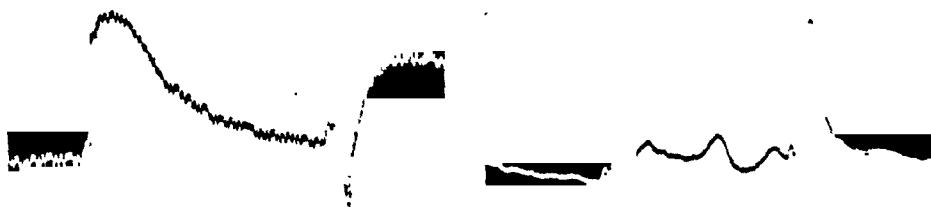


Fig. 2. Cat cocaineized (10 mgm./kgm.).

Epinephrine  
1 gamma

Postganglionic  
mesenteric fibres  
(deganglionated  
36 days before)  
approximately  
1 gamma equ.

Cat (no cocaine)

Postganglionic  
mesenteric fibres  
(deganglionated  
60 days before)  
approximately  
0.5 gamma equ.

Epinephrine  
0.5 gamma

(f) *Stimulation of the stellate ganglia* of 10 cats for 8 to 45 (average 16) minutes, with the coil distance of the inductorium varying from 7 to  $\frac{1}{4}$  cm., was accompanied by a marked tachycardia which, however, decreased gradually toward normal (fig. 3). At the conclusion of the stimulation period the heart muscle contained an average excess of +35 per cent of chromogenic material, as compared with 15 control hearts ( $P < 0.01$ , significant) (table 6). The d.s.r. was not significantly altered.

(g) *Stimulation of deganglionated cardiac sympathetic postganglionic fibres* of 10 cats for 4 to 10 (average 9) minutes with a coil distance of  $\frac{1}{4}$  cm. was accompanied by a distinct but decreasing acceleration in 3 animals while in one cat the heart rate remained unchanged and in 6 there was a retardation. The average curve (fig. 3) shows a sharp initial drop of the heart rate which is followed by a slight



acceleration, markedly below that of the control experiments. Only one out of the 10 cats showed a significant increase of chromogenic material in the myocardium (table 6). The average increase was not more than +4 per cent. The d.s.r. was slightly lowered.

**DISCUSSION.** The results obtained through colorimetric assay of epinephrine and related catechol compounds in adrenergic neurones and other tissues were in fair agreement with quantitative measurements made by Cannon and Lissák (5), by Lissák (6) and by von Euler (14) with biological tests (see table 1).

TABLE 6

*Epinephrine-like material in the heart muscle of cats under standard conditions, after stimulation of the stellate ganglia and after stimulation of the deganglionated post-ganglionic fibres of the cardiac sympathetic*

CONTROLS			STIMUL. STELLATE G.				STIMUL. DEGANGLIONATED POSTGANGL. FIBRES			
Cat no.	AC*	d.s.r.†	Cat no.	Minutes of stimul.	AC*	d.s.r.†	Cat no.	Minutes of stimul.	AC*	d.s.r.†
1	1451	1.18	16	8	1721	1.00	26	10	1285	1.00
2	1700	1.14	17	12	1882	1.09	27	10	1098	0.87
3	1590	1.09	18	12	1451	1.01	28	7	1768	1.01
4	1496	1.03	19	35	2080	?	29	10	1740	0.93
5	1377	1.12	20	45	1851	1.00	30	10	2212	1.00
6	1308	1.00	21	8	2613	1.04	31	10	1023	1.00
7	1544	1.07	22	10	2183	1.14	32	10	1275	1.06
8	1600	1.18	23	10	1707	0.99	33	10	1577	1.03
9	1585	1.01	24	10	2459	1.07	34	10	1541	1.05
10	1629	1.00	25	10	1586	1.09	35	4	1498	0.99
11	1029	1.18								
12	1339	1.05								
13	1824	1.06								
14	1335	1.11								
15	931	1.45								
Av. . . . .	1449	1.11		16	1953	1.05		9	1502	0.99
Diff. . . . .					+35%				+4%	

\* and † See table 2.

The adrenergic neurones (sympathetic ganglia and postganglionic fibres) contained relatively high concentrations of epinephrine and related compounds. Only small amounts were detected in the cervical vagus and sciatic nerves, obviously in proportion to admixed sympathetic fibres, while no such material could be found in somatic nerves which are free of sympathetic fibres.

The conception of a sympathetic neuro-secretion as outlined in the introduction is supported by the following of our own observations:

(1) The concentration of epinephrine and epinephrine-like material was consistently higher in the sympathetic ganglia than in the postganglionic fibres and extra-ganglionic sections of the thoracic sympathetic trunk.

(2) The range of variation of epinephrine and related compounds in sympathetic neurones was found to be much wider than in other tissues. This seems to be compatible with the assumption that varying stages of neuro-secretory activity existed in the different test animals at the time of their death when specimens were obtained for analysis.

(3) Severance of the connection between postganglionic fibres and their respective ganglionic cell bodies was followed not only by quantitative diminution but also by qualitative deterioration of the postganglionic sympathomimetic material, as concluded from its abnormal effect on the blood pressure of the cat

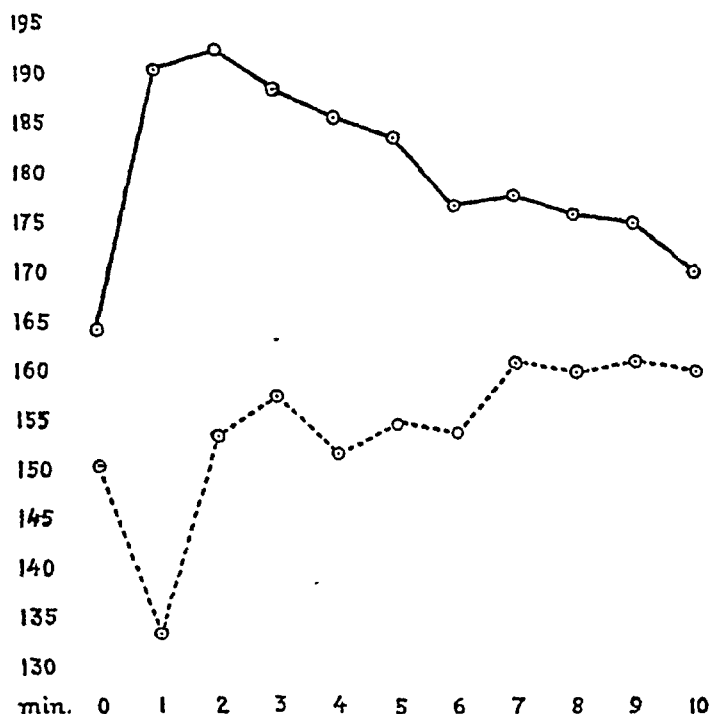


Fig. 3. Average heart rates during 10 minutes' faradic stimulation of:

(a) both stellate ganglia (10 cats) ———.

(b) postganglionic cardiac sympathetic fibres on both sides after resection of the stellate ganglia (10 cats) - - - - -.

The initial drop seemed to be due to spreading of the current to the vagus; the average increase of heart rate during the last minutes of (b) is attributable to the death of 2 animals with particularly slow heart rates after 4 and 6 minutes respectively.

and from the behavior of the d.s.r.. The fact that almost complete disappearance of chromogenic material from the deganglionated mesenteric sympathetic fibres was observed in only one out of eight deganglionated dogs may be ascribed to the remaining in situ of some scattered ganglionic cell groups near the coeliac ganglion. Lissák (6) mentions "disappearance" of the biologically tested epinephrine-like material from isolated postganglionic sympathetic fibres, however, without giving any quantitative data.

(4) Intense electrical stimulation of adrenergic neurones led to a gradual depletion of their content in sympathomimetic amines, especially if stimulation was continued for several hours and if isolated postganglionic fibres were stimulated.

This was concluded from the following findings: *a.* Diminution of epinephrine and related compounds in stimulated neurones, as manifested in the colorimetric results. *b.* Increase of epinephrine-like material in the mammalian heart during stimulation of the cardiac sympathetic (stellate ganglia). *c.* Nearly complete abolition of both the increase of epinephrine-like material in the mammalian heart and of the cardiac acceleration if the postganglionic fibres of the cardiac sympathetic were stimulated alone after removal of the stellate ganglia.

Further evidence of the adreno-sympathetic origin of epinephrine-like material in sympathetic-innervated tissues, found both with biological tests (5, 7, 14, 15, 16) and with biochemical methods (5, 8, 12, 14, 15) is the diminution or disappearance of this sympathomimetic chromogenic material after sympathectomy, without or with adrenalectomy (5, 15, 17). Sympathetic denervation of the heart as well as total sympathectomy were found to be accompanied by a marked diminution of chromogenic material in the heart and by a slowing of the heart rate (17).

Various organs contain much higher concentrations of epinephrine and epinephrine-like substances than could be explained alone by the sum of epinephrine-containing sympathetic nerve endings present in their parenchyma. Furthermore, much of this material found in sympathetic-innervated tissues is not identical with epinephrine proper (12, 14), not even in case of its accumulation following injection of large doses of epinephrine or following sympathetic stimulation (18). This seems to indicate that epinephrine, after having reached the effector cells either directly through discharge from sympathetic nerve endings, or from the adrenal glands and from remote sympathetic neurones by the blood stream, is being rapidly transformed inside the effector cells into biologically and chemically different but still closely related compounds with an intact catechol nucleus. Without the catechol nucleus preserved these products would not be detectable with the colorimetric method used (12).

The identity of this intracellular epinephrine-like chromogenic material with biologically active sympathomimetic amines is suggested by the approximate agreement of quantitative biological and colorimetric tests (table 1), as obtained by different investigators. These findings seem to support the views of Cannon and Rosenblueth (9) regarding the intracellular modification of sympathogenic epinephrine, resulting in the formation of sympathin inside the effector cells. Recent observations by von Euler (14) suggest that sympathin consists of amino- and ethylamino-bases of the catechol group.

#### SUMMARY

Colorimetric determination of epinephrine and closely related catechol compounds in adrenergic neurones and in sympathetic-innervated tissues yielded results which agreed satisfactorily with quantitative biological tests.

It was found that sympathetic ganglia contain relatively high concentrations of epinephrine and epinephrine-like compounds, that the concentration in postganglionic fibres is somewhat lower and that the latter diminishes or disappears after severance of the postganglionic fibres from their respective ganglion cells, and also during prolonged electrical stimulation.

Stimulation of sympathetic neurones was found to be followed by an increase, sympathetic denervation by a decrease of epinephrine-like chromogenic material in the respective effector cells.

The chromogenic material present in most sympathetic-innervated tissues proved to be different from epinephrine proper, even in case of its massive accumulation following epinephrine injection. It seems to derive both from local sympathetic epinephrine discharges and from adrenal medullary secretion.

These observations support the conception of a neuro-secretory activity of the sympathetic nervous system by which epinephrine and closely related catechol compounds are formed in the ganglion cells and flow down the axons under the influence of adequate stimuli to be transformed into sympathin within the effector cells.

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# EFFECT OF SYMPATHECTOMY WITHOUT AND WITH ADRENAL INACTIVATION ON THE CONCENTRATION OF EPINEPHRINE AND RELATED COMPOUNDS IN VARIOUS TISSUES

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The presence of epinephrine and of related epinephrine-like substances in tissues containing sympathetic adrenergic nerve endings has been demonstrated with biological and chemical methods (1, 2, 3, 4, 5, 6, 7, 8).

The probable origin of part of this sympathomimetic material in adrenergic neurones and its neuro-secretory discharge into the effector cells, where transformation of epinephrine into sympathin takes place, has been discussed in a preceding paper (9). Another part of the sympathomimetic material found in various tissues can be assumed to derive from adrenal medullary secretion and from remote neuro-secretory discharges through humoral transportation. Finally, local formation by scattered chromaffine cell groups has to be considered.

The following experiments were carried out in an effort to further investigate the sources of sympathomimetic material through determination of the effect of sympathetic denervation without and with adrenal inactivation on the concentration of epinephrine and related substances (sympathin) in various organs.

**METHODS.** All operative procedures were performed on male cats by the late Dr. J. P. Maes, partly in Burlington, Vt., partly in Hanover, N. H., whence the animals were transported alive to Burlington for chemical analysis.

Sympathetic denervation of the heart and the forelegs was carried out in two stages by removal of the lower cervical and stellate ganglia and of the thoracic trunks down to the 6th, 7th and 8th ribs. Total sympathectomy was carried out in four stages.

Adrenal "inactivation" consisted either of removal of one adrenal gland and demedullization of the other (two stages) without substitutive therapy, or of total epinephrectomy with implantation of desoxycorticosterone acetate pellets and forced feeding with addition of NaCl. Where total sympathectomy was combined with adrenal inactivation, the latter followed the former.

For chemical analysis the cats were killed with lethal doses of nembutal and the tissues were worked up immediately. The analyses were carried out with a modification of Shaw's (4) colorimetric method, the technicalities and the specificity of which have been discussed in previous publications (8, 9, 10), including the significance of the d.s.r. (denominator of specific ratio) for a rough evaluation of the qualitative composition of the total readings obtained.

<sup>1</sup> Doctor Maes died in Belgium on August 7, 1946.

Probability of statistical insignificance of the results obtained (P of Fisher's t-test) was kindly calculated by Dr. James Crow of Dartmouth College.

TABLE 1  
*Epinephrine-like substances in the heart muscle*

CAT NO.	DATE	AC*	d.s.r.†	CAT NO.	DATE	AC*	d.s.r.†	NUMBER OF DAYS AFTER LAST OPERATION
Controls				Sympathetic denervation of the heart				
1	10/27/44	1451	1.18	11	1/12/45	840	1.38	33
2	10/28/44	1700	1.14	12	1/12/45	893	1.12	33
3	10/31/44	1590	1.09	15	2/17/45	898	1.06	69
4	11/ 2/44	1496	1.03	18	2/19/45	705	1.15	70
6	11/21/44	1377	1.12	19	2/19/45	699	1.14	70
8	11/26/44	1308	1.00	Av.....		807	1.17	
9	1/12/45	1544	1.07	Diff.....		-44%		
10	1/12/45	1600	1.18	Total sympathectomy + adrenal inactivation‡				
13	2/17/45	1585	1.01	5	11/20/44	878	1.30	4
14	2/17/45	1629	1.00	7	11/22/44	922	0.87	7
16	2/19/45	1029	1.18	23	9/19/45	651	1.00	7
17	2/19/45	1339	1.05	25	9/20/45	808	1.48	41
20	9/18/45	1824	1.06	Av.....		815	1.16	
22	9/19/45	1335	1.11	Diff.....		-44%		
24	9/20/45	931	1.45	Plain adrenalectomy				
Av.....		1449	1.11	21	9/18/45	1645	1.20	61
				26	9/21/45	1555	1.10	52
				Av.....		1600	1.15	

\* The term AC (adsorbable chromogens) is being used here, as in previous publications, to indicate the total colorimetric readings, expressed in color units per gram of tissue. One color unit equals in color intensity 0.001 gamma of epinephrine.

† Denominator of specific ratio (see the preceding paper by Raab and Humphreys, p. 461).

‡ Removal of one adrenal gland and demedullization of the other in cats 5, 7; bilateral adrenalectomy in cats 23, 25.

RESULTS. *Heart.* In all of 5 cats with sympathetic denervation of the heart and in all of 4 cats with total sympathectomy plus adrenal inactivation (table 1) the readings were below the normal minimum, with an average diminution of 44 per cent below the normal average in both groups. These diminutions were statistically significant ( $P = < 0.01$  in both groups). The d.s.r. remained practically unchanged.

The results in 2 cats with plain adrenalectomy without sympathetic denervation were within normal range.

*Striated muscle of the left foreleg.* Thoracic sympathectomy (5 cats) as well as total sympathectomy plus adrenal inactivation (4 cats) (table 2) was followed by an average diminution of 27 and 31 per cent respectively. All readings were

TABLE 2  
*Epinephrine-like substances in the striated muscle of the left foreleg*

CAT NO.	DATE	AC*	d.s.r.†	CAT NO.	DATE	AC*	d.s.r.†	NUMBER OF DAYS AFTER LAST OPERATION
Controls				Sympathetic denervation of the heart				
1	10/27/44	779	1.16	11	1/12/45	643	1.66	33
2	10/28/44	988	1.25	12	1/12/45	520	?	33
3	10/31/44	1226	1.29	15	2/19/45	784	1.43	69
4	11/ 2/44	753	1.43	18	2/19/45	387	1.07	70
6	11/21/44	972	1.17	19	2/19/45	636	1.38	70
8	11/26/44	1023	1.46	Av.....		594	1.38	
9	1/12/45	781	1.27	Diff.....		-27%		
10	1/12/45	451	1.67	Total sympathectomy + adrenal inactivation‡				
13	2/17/45	801	1.12	5	11/20/44	773	1.08	4
14	2/17/45	838	1.11	7	11/22/44	686	1.50	7
16	2/19/45	556	1.00	23	9/19/45	397	1.35	7
17	2/19/45	756	1.18	25	9/20/45	379	1.51	41
20	9/18/45	1020	0.90	Av.....		550	1.36	
22	9/19/45	675	1.72	Diff.....		-31%		
24	9/20/45	561	1.67	Plain adrenalectomy				
Av.....		812	1.29	21	9/18/45	1177	1.22	61
				26	9/21/45	407	1.25	52
				Av.....		792	1.24	

\* and † and ‡ See table 1.

below the normal average. The diminutions were statistically of questionable significance ( $P = 0.07$  in both groups). There was no significant change of the d.s.r.

Plain adrenalectomy (2 cats) did not produce characteristic changes.

*Kidney, liver, spleen.* Total sympathectomy, combined with adrenal inactivation in 4 cats (tables 3, 4, 5) was followed in these organs by average diminutions of 23, 53 and 64 per cent respectively. Ten out of the 12 readings were below the corresponding normal minima. The respective Ps were 0.12 for the kidney (in-

TABLE 3  
*Epinephrine-like substances in the cortex of the kidney*

CAT NO.	DATE	AC*	d.s.r.†	CAT NO.	DATE	AC*	d.s.r.†	NUMBER OF DAYS AFTER LAST OPERATION
Controls				Total sympathectomy + adrenal inactivation‡				
1	10/27/44	1059	1.36	5	11/20/44	1474	1.04	4
2	10/28/44	1263	1.12	7	11/22/44	892	1.15	7
3	10/31/44	1590	1.23	23	9/19/45	731	1.13	7
4	11/ 2/44	1069	1.34	25	9/20/45	926	1.14	41
6	11/21/44	1315	1.02	Av.....		1006	1.11	
8	11/26/44	1782	1.09	Diff.....		-23%		
20	9/18/45	1698	1.08	Plain adrenalectomy				
22	9/19/45	1035	1.11	21	9/18/45	2030	1.35	61
24	9/20/45	983	1.20	26	9/21/45	803	1.07	52
Av.....		1310	1.17	Av.....		1416	1.21	

\* and † and ‡ See table 1.

TABLE 4  
*Epinephrine-like substances in the liver*

CAT NO.	DATE	AC*	d.s.r.†	CAT NO.	DATE	AC*	d.s.r.†	NUMBER OF DAYS AFTER LAST OPERATION
Controls				Total sympathectomy + adrenal inactivation‡				
1	10/27/44	1553	0.96	5	11/20/44	1918	1.01	4
2	10/28/44	3209	0.95	7	11/22/44	949	0.93	7
3	10/31/44	3889	0.90	23	9/19/45	1171	0.91	7
4	11/ 2/44	4341	0.86	25	9/20/45	959	1.00	41
6	11/21/44	1992	1.02	Av.....		1249	0.96	
8	11/26/44	2584	0.84	Diff.....		-53%		
20	9/18/45	3235	1.00	Plain adrenalectomy				
22	9/19/45	1711	0.97	21	9/18/45	4892	0.75	61
24	9/20/45	1651	1.04	26	9/21/45	923	1.11	52
Av.....		2685	0.95	Av.....		2907	0.93	

\* and † and ‡ See table 1.

significant), 0.02 for the liver (significant), < 0.01 for the spleen (significant). There was no significant change of the d.s.r. in either organ.



Results following plain adrenalectomy without sympathetic denervation (2 cats) were inconclusive.

*Adrenal cortex.* Demedullization of the adrenal cortex resulted in a sharp drop of the amount of epinephrine normally present there (table 6).

It is noteworthy that the time element seemed to be of no particular significance for the effect of sympathectomy, in that the diminutions after thoracic as well as total sympathectomy were about equally developed 7, 33, 41, 69 and 70 days after the respective operations. Only the one animal killed 4 days after total sympathectomy plus adrenal inactivation showed readings considerably nearer the normal average than the ones which were killed after longer intervals.

TABLE 5  
*Epinephrine-like substances in the spleen*

CAT NO.	DATE	AC*	d.s.r.†	CAT NO.	DATE	AC*	d.s.r.†	NUMBER OF DAYS AFTER LAST OPERATION
Controls				Total sympathectomy + adrenal inactivation‡				
1	10/27/44	1653	1.06	5	11/20/44	1183	1.12	4
2	10/28/44	2857	0.97	7	11/22/44	643	1.05	7
3	10/31/44	2445	0.95	23	9/19/45	880	1.06	7
4	11/ 2/44	1622	0.94	25	9/20/45	440	0.94	41
6	11/21/44	1857	0.95	Av.....		786	1.04	
8	11/26/44	2572	0.91	Diff.....		-64%		
20	9/18/45	3310	0.96	Plain adrenalectomy				
22	9/19/45	1997	1.07	21	9/18/45	2684	0.80	61
24	9/20/45	1595	1.01	26	9/21/45	440	0.94	52
Av.....		2212	0.98	Av.....		1562	0.87	

\* and † and ‡ See table 1.

Furthermore, it should be noted that total sympathectomy plus adrenal inactivation did not result in a more marked depletion of the heart and striated muscle of the foreleg than thoracic sympathectomy alone.

The heart rates in nembutal anesthesia (15 min. after intraperitoneal injection of 30 mgm. per kgm.) were markedly diminished in the sympathectomized animals, especially in those with adrenal inactivation, while plain adrenalectomy seemed to be less effective (table 7).

**DISCUSSION.** The salient features observed after thoracic sympathetic denervation, or total sympathectomy combined with adrenal inactivation, or plain adrenalectomy, were the following:

(a) Sympathetic denervation without or with adrenal inactivation was followed by a marked diminution but never by complete disappearance of chromo-

genic epinephrine-like material in the denervated tissues within 10 weeks. It was also followed by bradycardia.

(b) Adrenal inactivation (removal of one adrenal gland and demedullization of the other or total adrenalectomy) did not seem to materially enhance this diminution of chromogenic material in the denervated tissues nor did adrenalectomy per se (2 cats only) elicit characteristic changes in the tissues within 61 days (under treatment with desoxycorticosterone acetate).

(c) The degree of depletion of epinephrine-like material in the denervated tissues remained approximately constant within a time period of 1 to 10 weeks following the final operation.

(d) The chromogenic material remaining in the tissues after denervation with- or without adrenal inactivation did not show any grossly discernible qualitative chemical alterations compared with that present in the control specimens. This was concluded from the fact that the d.s.r. retained its characteristics, peculiar for each type of tissue, practically unchanged.

TABLE 6

*Epinephrine and epinephrine-like substances  
in the adrenal cortex*

			AC*	d.s.r.†
Controls			27,692	2.43
			10,859	2.32
Demedullated cortex	(4 days)		324	2.60
Demedullated cortex	(7 days)		1,012	1.07

\* and † See table 1.

TABLE 7

*Heart rates per minute*  
(All animals under nembutal anesthesia)

GROUP OF EXPERIMENTS	RANGE	AVER- AGE
Normal.....	144-228	166
Heart denervated.....	120-172	137
Total sympathectomy + adrenal inactivation†..	66-130	94
Plain adrenalectomy....	104-152	128

† See table 1.

This latter point speaks strongly against the possible assumption that the remaining material might be composed of some non-specific chromogenic substances, not belonging to the group of sympathogenic sympathomimetic catechol compounds.

Biological tests performed by Cannon and Lissák (5) with extracts made from hearts and livers after sympathetic denervation showed disappearance of the normally present epinephrine-like vasopressor and frog heart-stimulating properties of these extracts while other epinephrine-like effects, namely, those on the iris and nictitating membrane, were only weakened but not abolished by sympathectomy. Also the chromogenic effects of heart extracts, as tested with the method of Viale by Bacq (1) and with the method of Shaw by Cannon and Lissák (5) were only weakened but not abolished, in agreement with our own findings.

Despite the absence of major differences between the d.s.r. of normal and denervated tissues and between normal and deganglionated sympathetic fibres (9), it seems that the residual sympathomimetic amines both in denervated

tissues and in deganglionated degenerated nerve fibres are qualitatively different from the total material detectable under normal conditions, as evident from their abnormal biological effects on the blood pressure, etc. (5, 9).

It seems obvious that the losses of chromogenic material encountered in various tissues following sympathetic denervation are to be attributed to the partial or complete disappearance of such material from the degenerating postganglionic sympathetic fibres, as described in the preceding paper (9), and to the cessation of sympathomimetic discharges into the denervated tissues. The paralleling bradycardia, as well as observations made by White, Smithwick and co-workers (11) in radically sympathectomized hypertensive patients (normalization of the pathological electrocardiogram, even if the blood pressure remained elevated), and by one of us (10) in the heart of a sympathectomized patient whose myocardial concentration of epinephrine-like material was 88 per cent below the normal average, point in the same direction.

The question regarding the origin of the residual material found in the denervated tissues remains open to speculation. That it does not derive essentially from either the adrenal medulla or from remote epinephrine-discharging sympathetic neurones is suggested by the non-intensification of the depleting effect of local denervation (heart, muscles of foreleg) through combined total sympathectomy and adrenalectomy.

Consequently, one has to consider other intrinsic or extrinsic sources, such as:

- a. Continued local formation of sympathomimetic amines by scattered chromaffine cells (6, 12), as they have been described in the heart muscle by various authors (13, 14, 15).
- b. Continued influx from the brain which contains large amounts of a substance which, although not identical with epinephrine, seems to be related to it chemically and pharmacodynamically, and from the spinal cord. (A detailed study of this cerebrospinal material, the largest concentrations of which are present in the basal ganglia of the brain, is in progress.)
- c. Immobilization of a certain amount of pre-denervation material, deposited in the effector cells or in the neurilemma of the degenerated sympathetic nerve terminals (9) or in both.

Despite the inconclusiveness of the results obtained in 2 cats after plain adrenalectomy and treatment with desoxycorticosterone acetate, these latter experiments were not continued because the effect of adrenalectomy (without desoxycorticosterone treatment) had been studied previously by the one of us (16) in rats. The average diminutions of epinephrine-like chromogenic material (which in these older papers had been erroneously believed to include cortical steroids) were the following after an average survival period of 14 days: 22 per cent in the heart muscle, 19 per cent in the striated muscle, 33 per cent in the liver, 47 per cent in the spleen.

Altogether, the conclusion seems justified that the sympathomimetic amines present in various sympathetic-innervated tissues derive mainly from the supplying sympathetic neurones and also from adrenal medullary secretion. The origin of an additional fraction which seems to be pharmacodynamically different is not accounted for. Local formation through scattered chromaffine cells and/or influx from cerebrospinal sources appear to be possibilities.

## SUMMARY

Total sympathectomy, combined with adrenal inactivation, was followed by a marked diminution of the epinephrine-like substances present in the heart muscle, liver and spleen of cats. The changes found in striated muscle and kidney appeared less significant. In the heart and in the striated muscle of the foreleg mere thoracic sympathectomy produced changes of a magnitude equal to that resulting from total sympathectomy and adrenal inactivation. Bradycardia developed following the above-named operations.

Plain adrenalectomy with subsequent desoxycorticosterone therapy in two cats did not yield conclusive results but in a previous larger experimental series in rats this procedure (without desoxycorticosterone) had also been found to diminish the content of epinephrine-like material distinctly in various tissues.

The residual epinephrine-like material, remaining in the tissues after sympathectomy without and with adrenal inactivation, seems to be chemically essentially identical with the total material normally present but there are indications that it is altered regarding its pharmacodynamic properties. Possible sources of its origin are briefly discussed.

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# HEAT PRODUCTION IN RELATION TO BODY WEIGHT AND BODY SURFACE. INAPPLICABILITY OF THE SURFACE LAW ON DOGS OF THE TROPICAL ZONE<sup>1</sup>

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This paper records a series of investigations conducted by the writer in São Paulo, Brazil, on the minimal heat production in dogs of very different weights. Unexpectedly this minimum metabolism was found to be much more constant when referred to the body weight unit (more exactly to  $W^{0.90}$ ) than to the body surface ( $kW^{0.66}$ ) unit or to a 0.66–0.74 power of weight.

These results are therefore in complete opposition to those generally reported by other authors working on this subject.

The methods used and the results obtained by the writer are here described; a statistical treatment of the data is also given. Finally our results are discussed and compared with the data from the literature. An attempt is made to explain them by the climatic conditions under which our animals lived.

**EXPERIMENTAL PART. Animals.** The heat production was measured on 65 adult male dogs of mixed breeds, of varied colors and furs. Their weights ranged from 3 kgm. 400 to 31 kgm. 300. They were fed on cooked meat, corn meal and rice, and kept in very large rooms, so that they could move freely; sometimes they were also taken outdoors for exercise. Only animals in good health and showing weight constancy were used in the experiments.

**Climate.** The determinations were carried out in the city of São Paulo (23°35' South and 46°38' W, Greenwich). The city lies exactly on the Tropic of Capricorn, at an altitude of ca. 800 meters above sea level. The mean annual temperature is 17°.6C and the average relative humidity 79.1 per cent. The determinations were indifferently made in all seasons of the year.

**Methods of Heat Production Determination.** The minimum heat production per hour (minimum metabolism, total calories), was calculated from the  $O_2$ -consumption of the entire animal, measured at complete rest and 12–20 hours after the last meal. We used 4.8 as a fixed calorific value of a liter of consumed  $O_2$ , the respiration quotient obtained in short-duration experiments (6–20 min.) not being relied upon. The hourly calories per kilogram and per square meter were obtained by dividing the minimal heat production by the body weight and by the body surface, the latter being calculated by means of the Meeh formula ( $S = kW^{0.66}$ ), the value 0.107 being used for  $k$ , this being the mean of the more recent values proposed by Rubner (2) for dogs of different races.

The determinations were made on trained dogs and in anesthetized dogs. In

<sup>1</sup> A part of the results reported in this paper was presented as a thesis for professorship of Physiology in the Escola Paulista de Medicina (1).

many cases the same animal was experimented on both as trained dog and as anesthetized dog.

*Trained dogs.* Two different methods were used which gave concordant results: the confinement and the open-circuit methods.

a. *Confinement method.* The chamber used consisted in a cylindro-conical brass bell, the inferior edge of which is sunk into vaseline oil filling a trough surrounding a metallic base. There are two small glass windows on the upper cone of the bell which allow a close observation of the animal inside. The temperature of the air in the chamber is shown by a thermometer. Near the top of the cone there are two groups of stopcocks connected respectively with two pipes one of which opens on the upper part of the chamber and the other on the lower one. In order to render the gases of the chamber homogeneous, a system of two communicating flasks connected to the stopcocks of each group was used. The flasks contained water covered with a thick layer of vaseline oil. By rocking these flasks a certain amount of air may be introduced and at the same time an equivalent volume can be sucked with no important pressure alteration inside the chamber and without any leakage. The total amount of air in the system (chamber, communicating flasks and connecting tubes) was 137.813 liters. The volume of air breathed by the animal into the apparatus equals that total volume minus the volumes of the animal itself (the density considered as 1) and of a metallic cage. In view of its size, the apparatus can only be used for animals under 15 kgm. The animal is brought to the laboratory on the day before the test and is kept over night at a temperature of 20–22°C. Before the determination the dog, in the metallic cage, is put on the bottom of the apparatus and is there kept during the time necessary for a complete rest. Only then is the bell slowly lowered so that its inferior edge fits the base, and the only stopcock still opened is closed. The careful and noiseless handling of the mixing flasks starts at once and the animal is watched during 10 minutes. Very frequently the dog is slightly disturbed when enclosed in the chamber, therefore at least ten minutes after the dog returns to the resting condition (the mixing system working continuously) elapse before the experiment is started. By means of a second system of communicating flasks of small volume (also previously kept in motion), in connection with two stopcocks belonging to different groups, a sample of the air is rapidly collected. We call this sample *initial air*. A stopcock is next rapidly opened and closed in order to equalize the pressures inside and outside the chamber. This moment marks the beginning of the measuring of the metabolism and the temperature in the interior of the chamber and the atmospheric pressure are read. These data were used for the reduction of the chamber volume to 0°C. to 760 mm. pressure and to dryness. The mixing of the air is continued uninterruptedly till the end of the determination. The determination lasts, as a rule, 15 minutes. During the last 30 seconds of the test a sample of air is rapidly collected for analysis in one of the mixing flasks. By comparing the composition of the initial and final airs (the apparent variation in N being taken into account) we calculated the O<sub>2</sub> consumption per hour and the volume of air respired, the exact duration of the determination being known.

TABLE 1  
Heat production of 65 adult male dogs

[illegible]

TABLE 1—*Concluded*

DOGS NO.	BODY WEIGHT*	BODY SURFACE†	TOTAL/CAL./HOUR		CAL./KGM./HOUR		CAL./SQ.M./HOUR	
	(kgm.)	(sq. m.)	Trained	Anesthetized	Trained	Anesthetized	Trained	Anesthetized
16	17.670	0.7260		32.23		1.82		44.39
33	18.200	0.7400		27.73		1.52		37.47
29	19.800	0.7830		26.85		1.35		34.29
17	19.870	0.7840		27.56		1.39		35.15
4	20.000	0.7880		31.63		1.58		40.13
63	20.600	0.8049	31.88	34.58	1.55	1.68	39.60	42.96
30	22.200	0.8450		34.15		1.54		40.41
28	22.800	0.8600		34.98		1.54		40.67
61	22.800	0.8600	30.12		1.32		34.89	
19	23.470	0.8770		35.44		1.51		40.41
18	23.600	0.8804		30.25		1.28		34.36
48	24.200	0.8983	31.84	32.81	1.31	1.42	35.45	37.80
51	24.800	0.9130	33.93	36.67	1.37	1.48	37.16	40.17
35	25.000	0.9148		35.72		1.43		39.05
50	25.200	0.9228	34.23	36.38	1.42	1.50	38.10	40.50
47	26.000	0.9422	39.15	35.61	1.48	1.37	41.13	37.88
58	26.600	0.9567	42.77	38.38	1.61	1.44	44.71	40.12
49	27.600	0.9805	37.30	37.51	1.35	1.31	38.04	37.36
26	30.100	1.0350		43.55		1.44		42.08
25	31.300	1.0627		44.25		1.41		41.64

\* In the cases where two determinations are presented the weight at the time of only one determination is given.

† Calculated by  $S = 0.107W^{2/3}$  ( $W^{2/3}$  was obtained by  $\frac{2 \log. W}{3}$  or  $0.667 \log. W$ ).

Twelve trained dogs, below n°. 47 in table 1, were investigated by this method. It was required for this experiment that the animal remained perfectly still, crouching, with the head leaning on the bottom of the cage or on its own legs, as if sleeping. This condition is obtained by the previous repeated training of the animals. Any noise is likely to disturb the dog and should be completely avoided. For this reason the determinations are carried out in the morning when the dogs are still sleepy and the building silent. Another important factor is the temperature; it is maintained between 18 and 23°C. in the chamber. Above 24°C. the metabolism increases as a consequence of the disturbance and of the increased respiration rate of the animal. Furthermore, the amount of carbon dioxide in the interior of the chamber did not reach 2 per cent by the end of the determination. Only in two instances it reached 3 per cent, but this concentration is also known not to increase the metabolism.

b. *Open-circuit method.* It was possible, when experimenting on a pains-takingly trained lot of dogs, in perfect rest, to employ masks (similar to those used for humans) and Chaveau-Tissot valves, permitting to inspire outdoor atmospheric air and to collect the expired air in a Tissot spirometer. The whole system worked always under control, no leakage having been allowed. Of course



the training of the dogs and the determinations were made in a perfectly silent room. With the mask on, the untied animal stood for 20–30 minutes in a completely relaxed condition, lying on its side, as if sleeping. Only then the whole of the air expired was collected during six minutes and measured in a calibrated spirometer. The volume was reduced to N.T.P., samples were taken for analysis and the  $O_2$  consumption calculated. Nineteen trained dogs, of very different weights (numbered from n° 47 upwards in table 1) were submitted to these tests. Each of the results represents the lowest value of two determinations made on different days, but not differing more than 12 per cent. Very frequently both determinations agreed very closely and the difference was only of a few per cent, these results being quite comparable to those obtained when man is the patient. This method provided results that agreed well with those yielded by the confinement method. The determinations were made at a temperature between 18° and 24°C., the dogs were continuously under direct observation and shivering or reactions against cold did not occur. An attempt was made to measure metabolism at 28°C., which according to some writers corresponds to the thermic neutrality for dogs. However, we always recorded, at 24°C., a pronounced acceleration of the respiration rate which increased progressively and came to a great value at 27°–28°C. We did not, evidently, think of measuring minimum metabolism under such conditions.

*Anesthetized dogs.* The methods we have just described are very involved, chiefly owing to the fact that the animals seldom keep themselves perfectly still so that the determinations are frequently lost. Furthermore a certain number of dogs are not fitted for these tests. Anesthetized dogs were then used for the determination of the metabolism. An excellent anesthesia was obtained by injection of 0.30–0.40 cc. of Dial (Ciba) per kgm. intraperitoneally, immediately after administrations of morphine chlorhydrate (10 mgm. per kgm., subcutaneously). About 15 minutes after these injections the animal is perfectly anesthetized. A tracheal cannula is inserted, or a mask is fitted on the muzzle, and connected with the Chauveau-Tissot valves, and the expired air is directed to the Tissot spirometer. If necessary the animals are warmed up so that the rectal temperature, observed before the induction, is exactly maintained during the determination. A repose of half an hour, after the operation, is allowed and the procedure described in the preceding section is carried out.

*Air analysis.* All the air samples were analysed in a portable Haldane air-analysis apparatus. In order to calibrate the burette and control our technic, numerous analyses of the atmospheric air were made and repeated during the course of our work. Results of great constancy were always obtained. The maximum error of the combined percentage of  $O_2$  and  $CO_2$  did not exceed, in our determinations, 0.03 per cent of the theoretic percentage.

**RESULTS.** Table 1 shows the results of the heat production of 65 dogs the weights of which ranged from 3.400 to 31.300 kgm. and the cutaneous area, as calculated from Meeh's formula, from 0.24 to 1.04 sq. m. The dogs used twice, i.e., trained and anesthetized, sometimes had somewhat different weights at the time the two determinations were made. Only one of the weights is given in

table 1, but the calculations were always based on the weight at the time of the experiment.

The inspection of table 1 shows:

a. That heat production is not appreciably altered by the dial-morphine anesthesia in the doses used.

b. That the number of calories per surface unit clearly increases in accordance with the increase of the weight of the animals.

TABLE 2  
Mean heat production of 65 dogs  
(according to their weights)

WEIGHT RANGE  <i>kgm.</i>	CAL./KGM./HOUR		CAL./SQ.M./HOUR	
	Trained	Anesthetized	Trained	Anesthetized
3.500- 7.000	1.79	1.64	28.00	26.54
7.000-14.000	1.62	1.60	32.58	32.54
14.000-31.300	1.49	1.51	39.11	39.06

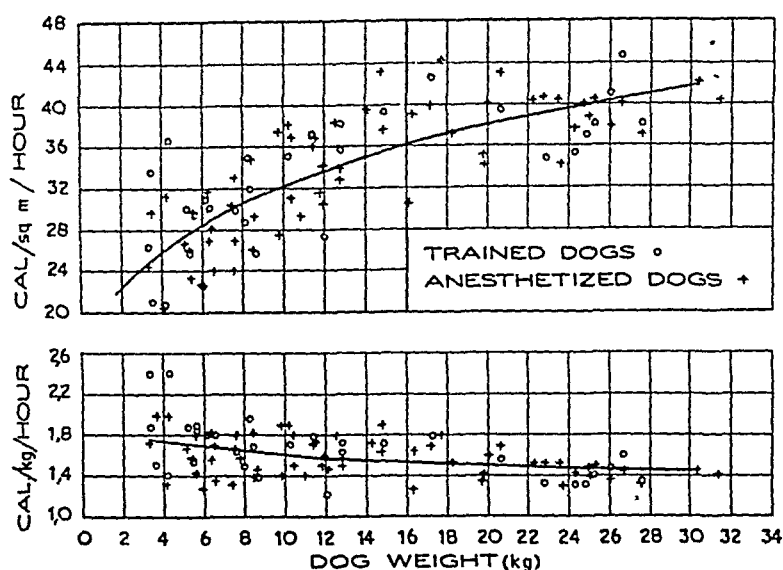


Fig. 1. Minimum metabolism per kilo and per square meter in dogs of different weights  
Calories per kilogram hour and per square meter hour plotted against total weights.

c. That the number of calories per weight unit is kept much more constant.

These results are clearly seen in table 2 which gives the mean heat production per weight and per surface units as calculated for three groups of dogs, the heaviest dog of each group being approximately double the weight of the lightest. Three different levels of heat production per square meter can be recognised. The heavier the group the higher the level.

On the contrary in all groups of dogs the heat production per kilogram is nearly the same so that only a level may be taken for all the dogs.

In figure 1 the calories per kilogram and per square meter are plotted against

the weights. The theoretical line, using the values of calories per weight and surface units, calculated by the method of least squares as explained in the next paragraph, are also shown.

In the case of the calories per kilogram the theoretical line is nearly parallel to the abscissae, while the line representing the calories per square meter shows an increase with the increasing weights. The greater constancy of the heat production per kilogram is thus plainly visible.

We are greatly indebted to Dr. A. C. Andrade who made the statistical calculations reported in the next section and to Dr. A. A. Bitancourt for his suggestions in connection with the statistical treatment of the data.

*Statistical treatment of the data.* In the first place a test was made of the significance of the mean, for 25 dogs, of the difference between the calories produced by the same dog, first as trained and after as anesthetized dog. This mean is 0.137 and its standard error is 0.228, so that the corresponding  $t$  value is 0.6, well below the significant level. This shows that the caloric production is not

TABLE 3

*Coefficients of regression calculated by the method of least squares from the data in table 1*

DOG	NUMBER	COEFFICIENT OF REGRESSION	STANDARD ERROR OF COEFFICIENT OF REGRESSION
Anesthetized.....	59	0.9229	0.0242
Trained.....	31	0.8790	0.0369
All dogs.....	90	0.9037	0.0211

significantly different when it is measured in the same dog, according to the two methods.

Next the exponent of the weight in the equation

$$C = aW^b$$

was calculated by the method of least squares, using the logarithms of the number of the total calories per hour and of the weight in kilograms, so that

$$\log C = \log a + b \log W$$

The exponent  $b$  in the first equation is the coefficient of regression in the second one and was obtained for the two groups of experiments, i.e., anesthetized and trained dogs respectively. It is given in table 3, together with its standard error.

Another proof that the two methods used did not give significantly different results as given by the  $t$  test of the difference of two coefficients of regression obtained with the trained and with the anesthetized dogs. The difference is not significant and the results have therefore been pooled so as to give a common coefficient for all the data, i.e., 0.9037, or approximately 0.9.

The equation for the whole of the data is

$$\log C = 0.3010 + 0.9037 \log W$$

with an error of estimate of  $\pm 0.0543$

It corresponds to the equation

$$C = 2.00 W^{0.9}$$

with a percentage standard error of  $+13.3$  per cent and  $-11.7$  per cent.

The  $t$  test shows that the coefficient of regression 0.9037 differs significantly from 1, i.e., from the exponent of the weight which is required if the number of calories is strictly proportional to the weight. Therefore it also differs significantly from 0.667 which corresponds to the equation in which the number of calories is related to the  $\frac{2}{3}$  power of the weight or to the body surface according to the Meeh formula.

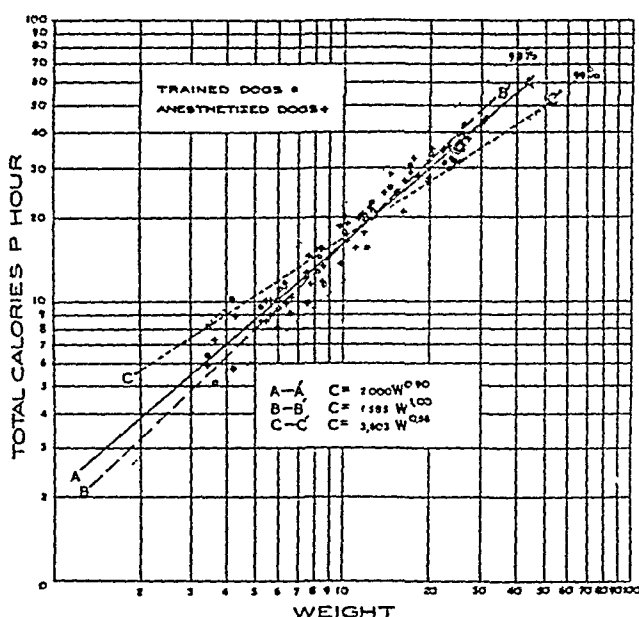


Fig. 2. Minimum metabolism and body weights. Total calories per hour plotted against weight on the logarithmic scale.

Figure 2 shows these results graphically. It is seen that while the regression line with the coefficient 0.903 fits the data adequately, this is not so for the line with coefficient 1 and much less so for that with the coefficient 0.667. Figure 2 also shows the lines corresponding to the 99 per cent level of the error of estimate.

Using the actual data instead of their logarithms, two different regression lines were adjusted (fig. 3). A linear regression line, corresponding to the equation

$$C = 2.302 + 1.3682 W$$

and a curvilinear regression line of the second order, or arc of parabola with the equation

$$C = 0.281 + 1.819 W - 0.014 W^2$$

An analysis of variance of these lines is shown in table 4.

The *F* test shows that the variance of the parabola differs significantly from the residual variance and therefore that the linear equation is not so satisfactorily adjusted to the data as the second degree equation is.

Statistically the data can be satisfactorily represented by two different equations:

$$C = 2.00 W^{0.90}$$

and

$$C = -0.281 + 1.819 W - 0.014 W^2$$

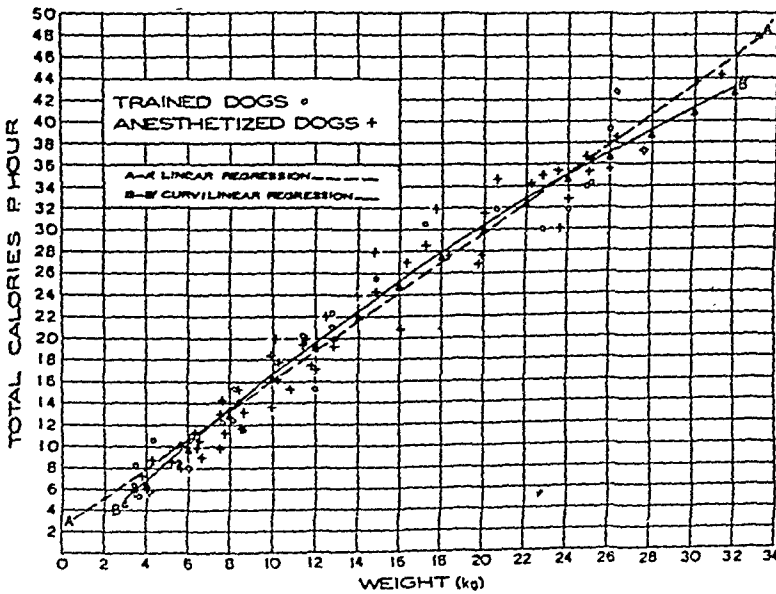


Fig. 3. Minimum metabolism and body weight. Total calories per hour plotted against weight on the arithmetic scale.

TABLE 4  
*Analysis of variance of the line and curvilinear regression lines*

SOURCE OF VARIATION	SUM OF SQUARES	D.F.	VARIANCE
Straight line about mean.....	10241.1984	1	
Parabola about straight line.....	46.6464	1	46.65 ++
Residual data about parabola .....	381.6783	87	4.39
Total.....	10669.5231	89	

No choice can be made of these equations based on statistical considerations because the deviations of the observed data from the values calculated from these equations do not differ significantly as shown by a comparison of the respective variances 4.553 and 4.288.

From a physiological point of view, however, it is obvious that no satisfactory interpretation can be given of the second degree equation whereas the equation

with the exponent 0.9 of the weight can be accounted for as will be explained in the next section.

DISCUSSION. The literature about heat production in relation to body weight and body surface is very extensive. Excellent reviews on the subject have been published by Rubner (2, 8), Harris and Benedict (3), Grafe (4), Lusk (5), Lehmann (6), Couto e Silva (7), Mitchell (9), Bohnenkamp (10), Kleiber (11), Du Bois (12), Benedict (13) and Brody (14).

In short it may be stated that the authors may be divided in two groups, viz.:

a. Those who found constancy of heat production per unit of the body surface ( $kW^{0.66}$ ) and think that the Rubner-Richet surface law is a thermal law and has a causal significance. The heat production would be adjusted to the heat loss through the skin area, in order to maintain a constant temperature in homeotherms.

b. Those who found that heat production is a function of weight to the 0.66–0.74 power (the power varying according to the different authors) and maintain that the surface law is only a coincidence. They admit that the metabolism is related to other factors which vary with  $W^{0.66-0.74}$ , such as active protoplasmic mass, blood volume, size of organs, cross section of trachea and cross section of aorta, etc.

Recently Benedict (13) with his extraordinary large experience in the subject considered futile any attempt to establish a relationship between heat production and body surface or body weight at any power so far proposed by the authors. He thinks that the variability of heat production is dependent on gross differences in the body make-up of animals of the same or of different species.

Only the intra-specific aspect of the subject, especially the data obtained on dogs, will be considered here.

Kunde and Steinhaus (15) tabulated the data obtained by Rubner, Lusk and Du Bois, Boothby and Sandiford and by themselves in a total of only 32 dogs. From the results of Rubner, Lusk and Du Bois as well as of Kunde and Steinhaus, Brody (14) found 0.6 for the power of weight.

To those results may be added those of Richet (16 and 17) (in spite of their too high values) and of Slowtsoff (18), which also agree with the surface law.

As a matter of fact it is rather surprising that so few experiments have been made on dogs in order to verify the applicability of the surface law within one animal species. It is well known that dogs are especially adequate to solve the problem as they show when adult much larger differences of weight than any other animal.

We do not know of any investigations made on dogs under tropical or sub-tropical conditions with the purpose of verifying the surface law, except our own.

Our results showing in dogs a much more constant heat production to weight unit (nearly constant to  $W^{0.90}$ ) than to body surface ( $kW^{0.66}$ ) or to  $W^{0.66-0.74}$ , are in sharp discrepancy with all data found in the literature.

The 0.90 power of the weight can be interpreted as representing the metabolic active weight of the animals, the animal's total weight increasing a little more

rapidly than the metabolic active weight. In other words, the metabolic inactive tissue can be thought to be comparatively more abundant in the large dogs than in the small ones. This would be the case if the gut, subcutaneous tissue, fat, fur, mineral contents of the bones, represent a larger percentage of the total weight in the larger animals.

In order to explain the antagonism between the results obtained by other authors and our own we should consider the different climatic conditions under which the experiments have been made. An interpretation can be made on the basis of the remarkable general theory on basal metabolism proposed in 1919 by A. Osorio de Almeida (19) to explain the influence of the tropical climate on the basal metabolism of man.

All the investigations on the problem, carried out so far on dogs have been made in cold climates (Berlin, Paris, New York, Chicago). The temperature differences between the animal surface and the surrounding milieu being usually very large in cold climates the heat loss through the surface may be a dominant factor in determining the minimal heat production. In our warmer climate this temperature difference is smaller, so that the surface heat loss would not influence the minimal heat production. This minimal heat production would then show itself proportional to the body weight.

Therefore, the earlier results being true, we could say that if the law is good only under cold climates, it has a thermic significance and expresses a causal relationship between heat loss through the surface and heat production.

It seems, however, that the number of well controlled experiments in dogs in cold climates is not sufficiently great and that a reinvestigation of the subject must be made in such climates before the above interpretation can be definitely accepted.

#### SUMMARY

The heat production of 65 dogs (from 3.400 to 31.300 kgm.) was determined by the  $O_2$  consumption method. Thirty-one determinations were carried out on trained dogs under basal conditions and 59 on dial-morphine anesthetized dogs. The determinations were made in São Paulo, Brazil.

The data were submitted to statistical treatment.

The minimal heat production was found to be much more constant per kilogram than per square meter and can be expressed by the equation

$$C = 2.00 W^{0.90}$$

The above results are in opposition to those of all other investigators who have always found the heat production of dogs to be more constant when referred to a 0.66 – 0.74 power of the weight than to the body weight itself.

The results obtained are discussed and compared with the data from the literature.

Although feeling that the reinvestigation of the heat production of dogs in cold climates is needed, the writer presents an interpretation of the divergent results, based on the different climatic conditions under which he worked.

This interpretation would support a view that the Rubner-Richet surface law, being applicable only in cold climates, has a causal, thermal significance.

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# EFFECTS OF HIGH OXYGEN TENSIONS UPON THE CARBON DIOXIDE PRODUCTION OF SKELETAL MUSCLE AND OTHER TISSUES OF THE FROG

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Paul Bert (1), who first described O<sub>2</sub> poisoning, recognized the metabolic effects of high O<sub>2</sub> tensions. He found that in rats and frogs the O<sub>2</sub> consumption and the CO<sub>2</sub> production increased to a maximum when the O<sub>2</sub> tension was about 0.4 atm., and then decreased at higher tensions. At the tensions necessary to elicit convulsions a definite drop in body temperature preceded the seizure. Others, also, have found a decrease in O<sub>2</sub> consumption (2, 3), CO<sub>2</sub> production (4, 5), and temperature (2, 4, 6) in animals subjected to O<sub>2</sub> at high pressure. Gersh (7), however, considers a fall in temperature a sign of a moribund state in the cat. Baker's yeast showed a very sharp decline in CO<sub>2</sub> output under O<sub>2</sub> at 3½ to 10 atm. pressure (8). There are a few records of increased O<sub>2</sub> consumption or CO<sub>2</sub> production. Bert found such increases at tensions between 0.2 and 0.4 atm. O<sub>2</sub> in rats, frogs, and himself. Bean (3) noted increased O<sub>2</sub> absorption in some dogs at 4 to 5 atm. O<sub>2</sub>; and Behnke (9) found increased O<sub>2</sub> consumption in men at 1 atm. O<sub>2</sub> as an early response to breathing O<sub>2</sub> at supranormal tensions.

Isolated tissues as well as intact organisms have shown susceptibility to poisoning by O<sub>2</sub>. Bert (1) observed that "muscular contractility, motor nerve excitability, and the rhythmic action of the nerve ganglia of the heart stop much sooner in oxygen under high tension than under normal pressure with ordinary air". Hill and Macleod (4) found that frog sartorii, but not thick gastrocnemii, after an hour in O<sub>2</sub> at 50 to 60 atm. pressure, gave a record resembling that of a fatigued muscle. Bean and Bohr (10), also working with frog sartorii, observed that 5-6 atm. O<sub>2</sub> caused an initial increase in contraction height with a decrease 1½ to 9 hours later. The treppe phenomenon disappeared at the time when decrease in contraction height appeared. A certain amount of permanent damage to the tissue was indicated by the facts that decompression to atmospheric pressure gave only partial recovery, and that recompression not only failed to cause an initial increase in contraction height, but brought about a decrease sooner than the first compression. Isolated frog hearts (4, 11) and smooth muscle of rabbit (12, 13, 14) gave unmistakable evidence of being poisoned by high O<sub>2</sub> tensions.

This study was undertaken to determine the effect of O<sub>2</sub> at high tensions upon the respiration (as indicated by CO<sub>2</sub> production) of an isolated tissue. The experimental work was done during 1941-1943 and reported in abstract form (15).

<sup>1</sup> On leave of absence 1940-1943 from the Department of Biology, Russell Sage College Troy, New York.

The apparatus described in this paper made it possible to follow the  $\text{CO}_2$  production of a tissue before, during and after its subjection to high  $\text{O}_2$  pressure without the complications incident in the usual manometric or volumetric methods.

In a careful study of the effect of high  $\text{O}_2$  tensions upon the metabolism of isolated mammalian tissues, Stadie, Riggs and Haugaard (14, 16, 17) found a decrease in  $\text{O}_2$  consumption (determined manometrically during the exposure of the tissue to high  $\text{O}_2$  pressure) but ordinarily no significant change in the R.Q. (calculated on the basis of a single  $\text{CO}_2$  determination for the experimental period). In brain, at least, the return to atmospheric pressure failed to check the fall in  $\text{O}_2$  consumption begun under high  $\text{O}_2$  pressure.

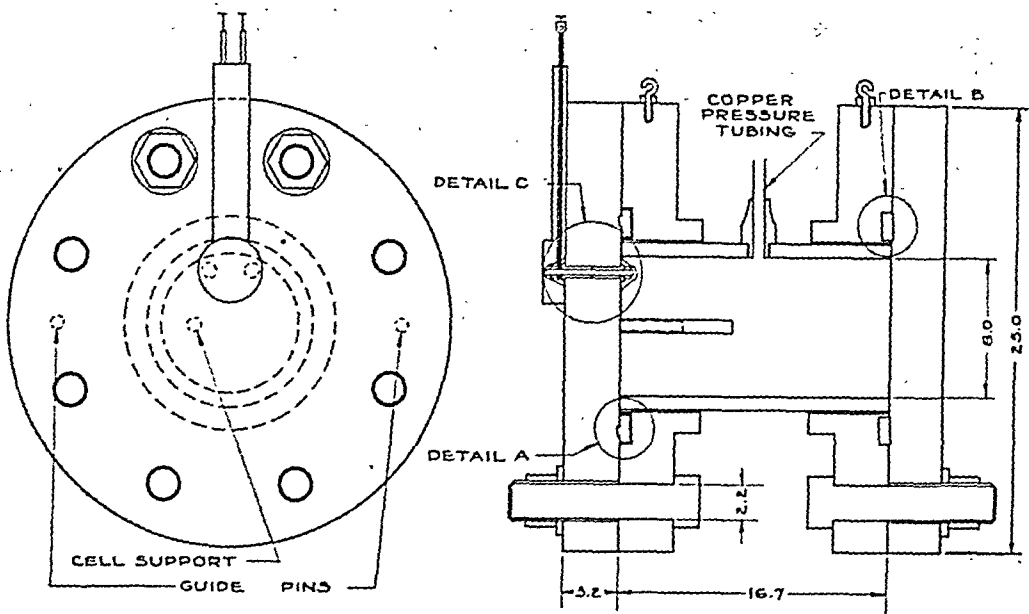
**METHOD.** The steel pressure chamber<sup>2</sup> (fig. 1), used for subjection of tissues to increased atmospheric pressure, was a cylinder onto each end of which was screwed a flange. End pieces similar to the flanges in diameter and in thickness were bolted to them. A lead gasket (fig. 1, detail B) made a pressure-tight junction between one of the flanges and one end piece which was bolted permanently in place. A circular groove in the flange of the open end, lined with steam-fitter's packing and sealed with a mixture of beeswax and rosin (fig. 1, detail A), held a brass gasket the free edge of which pressed into the steel end piece at a ground joint. The removable end was fitted with a pin which provided rigid support for a conductivity cell. Through this end piece ran two brass bolts insulated by tightly fitting fiber casings, the ends of which were flanged to act as insulating discs at the surfaces of the end piece (fig. 1, detail C). From the inner end of each brass bolt ran a fine enamel-covered copper wire to one of the mercury wells of a conductivity cell. On the outer surface, wires leading from the rods were protected from the water by rubber tubing and metal housing and were then connected to an electrical bridge by shielded wires. The inner and the outer connections of the brass rods with the copper wires were covered with a beeswax and rosin mixture, which served to insulate them as well as to make the end piece pressure-proof. Gas entered the chamber through a copper tube fitted with a pipe connection which was screwed into the cylinder itself. The joint was sealed with pipe-joint compound. The whole pressure chamber was suspended in a water bath by steel cables in such a way that either end could be raised or lowered by the operation of a small windlass.

For the determination of the  $\text{CO}_2$  production of isolated tissues the conductivity method, as described by Fenn (18), was used. This method depends upon the absorption of  $\text{CO}_2$  by  $\text{Ba}(\text{OH})_2$  with the precipitation of  $\text{BaCO}_3$  and the measurement of the concomitant decrease in the conductivity of the solution. Through the cover of the wide-necked conductivity cell (fig. 2 A) ran an inlet tube which extended nearly to the bottom of the cell. This served the double purpose of forcing any gas leaving the flask to pass close to the surface of the  $\text{Ba}(\text{OH})_2$  solution and of providing attachment for the silver wire support on which the tissue rested. Below the silver wire was a piece of glass cloth which prevented the spattering of the tissue by  $\text{Ba}(\text{OH})_2$ . The inlet tube was extended outside the cell by a few centimeters of capillary tubing of 2 mm. bore, which was intended to reduce to a minimum any exchange between gas inside and gas outside of the cell. Attached to the capillary tube by the shortest possible rubber connection was a soda lime tube (fig. 2 C) which absorbed a large part of the small amount of  $\text{CO}_2$  in the gas entering the cell after the cell had been closed. A second cover (fig. 2 B) with a reservoir permitted pouring a reagent onto a tissue while both were subjected to increased pressure in the bomb. For this the tissue lay in a small glass container which hung by silver supports above the  $\text{Ba}(\text{OH})_2$  solution.

The diffusion of phenol red from water in the flask to water in the mercury wells was used successfully in testing for leaks around the electrodes. Changes in the conductivity of the

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<sup>2</sup> The pressure chamber was designed by Prof. W. O. Fenn with the assistance of Prof. W. J. Conley.



PRESSURE CHAMBER  
DIMENSIONS IN CENTIMETERS

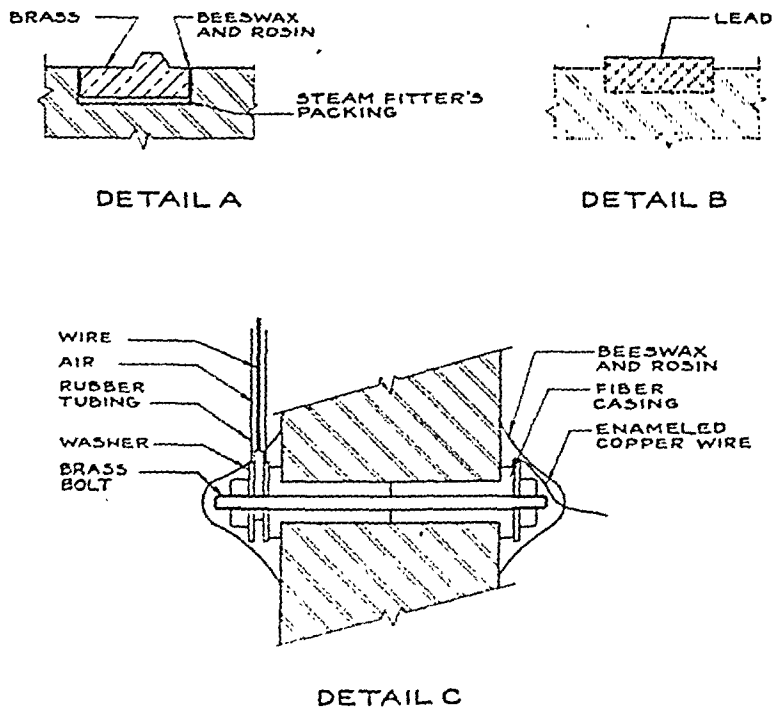
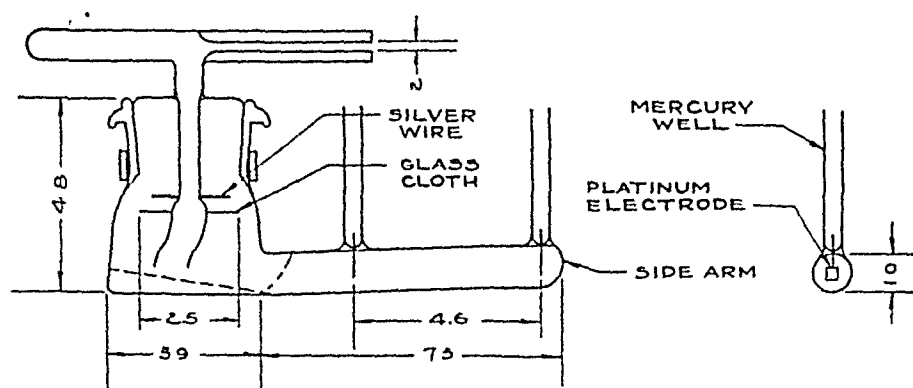
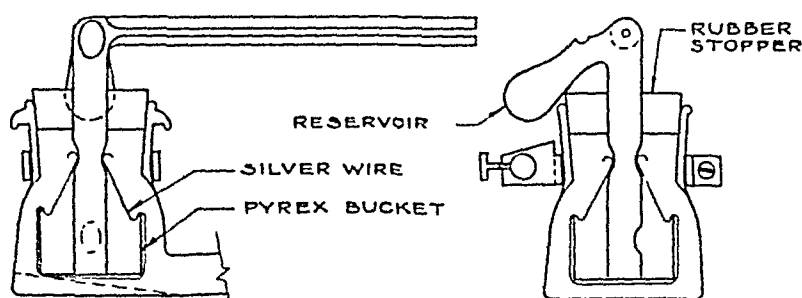


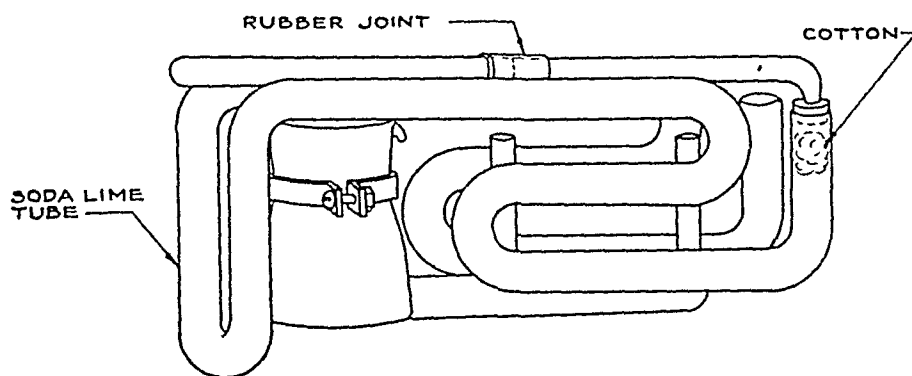
Fig. 1. Pressure chamber as described in the text. Dimensions in centimeters. Upper right, longitudinal section of closed chamber. Details of circled regions are shown, enlarged, below. Upper left, outer surface of removable end.



A



B



C

Fig. 2. Conductivity cell. Dimensions in centimeters. (A) Longitudinal section through cell showing silver wire support on which tissue rests, and glass cloth shield below. Broken lines indicate the position of the surface of  $\text{Ba}(\text{OH})_2$ : (at right) when cell is in reading position with solution filling the side arm; (at left) when cell is tilted back, exposing a large surface of the solution to the gas in the cell. (B) A second cover, for the cell shown in A, has a glass reservoir which may be filled through the small hole at its neck. A tight rubber band covers the hole leaving the capillary tube the only inlet to the cell. The tissue lies in the pyrex bucket which fits loosely against the end of the inlet tube. The bucket hangs high enough so that the  $\text{Ba}(\text{OH})_2$  solution can drain freely into the side arm of the cell. (C) Soda lime tube in position.

$\text{Ba}(\text{OH})_2$  solution were detected by use of a suitable A-C bridge. Two cells were used ordinarily, one within the pressure chamber and the other immersed directly in the water bath at atmospheric pressure.

The temperature of the large water bath varied over a maximum range of  $0.035^\circ\text{C}$ . within one heating cycle. This value includes a fleeting peak, the fluctuation of the range for the greater part of the cycle being  $0.003^\circ\text{C}$ . to  $0.020^\circ\text{C}$ . with  $0.005^\circ\text{C}$ . the mode. The simultaneous fluctuations in the cell itself inside the bomb must have been negligible.

All measurements were corrected for the change in conductivity due to the absorption of a small amount of  $\text{CO}_2$  which either passed through the soda lime tube, when pressure was increased, or may have diffused through the stopcock grease later. Correction was also made for similar small changes in conductivity which occurred in the absence of tissue during decompression.

To test the measurement, with and without pressure, of the  $\text{CO}_2$  output of some system other than living tissue, strips of a mixture of agar and  $\text{CaCO}_3$  on a cotton cord base were coated with acid agar and then mounted in the cells. After the usual correction, there was no difference between pressure and no-pressure samples and, in the pressure runs, no indication of the point at which pressure was applied. Thus there was no perceptible interference by increased  $\text{O}_2$  pressure with the diffusion of  $\text{CO}_2$  from the sample or with its absorption by the  $\text{Ba}(\text{OH})_2$  solution.

Frog tissues were used exclusively because of their ability to maintain a relatively constant respiratory rate over a period of many hours. The major part of the work was done on frog *sartorii* because their thinness favors the diffusion of gases into and out of the tissue. One muscle was run in the pressure chamber and the matching muscle, from the same frog, was run in a second cell without pressure, as a control. Skin, liver, lung, nerve and kidney were used in a few experiments for comparison with muscle.

After the pressure chamber had been closed and lowered into the bath, a minimum of an hour was allowed for the achievement of temperature equilibrium. A control period of one or two hours followed during which the initial or resting rate of  $\text{CO}_2$  production by the tissue was determined. The increase in pressure was brought about rather slowly, usually at a rate of  $\frac{2}{3}$  to  $2\frac{2}{3}$  atm. per minute, to allow maximum absorption of  $\text{CO}_2$  by the soda lime. The total pressure in each case was recorded as gauge pressure plus one atmosphere. At the close of a high pressure period gas was ordinarily released rapidly. When the  $\text{CO}_2$  production was to be followed after decompression, the gas was released slowly over a period of 45 to 60 minutes. Readings were made customarily at 30 minute intervals except for a 60 minute period at the beginning of compression.

**RESULTS.** The  $\text{CO}_2$  production of the frog *sartorius* under 8 to 35 atm.  $\text{O}_2$  changes according to a pattern already observed in other effects of high  $\text{O}_2$  tensions upon organisms and their tissues. It increases during the first hour at the raised pressure and later decreases to a rate lower than the initial one. The  $\text{CO}_2$  production of control muscles remains essentially constant for hours (fig. 3A).

Although no frogs with obvious red leg infections or with parasites visible in the muscle were used, there was a wide scatter in the  $\text{CO}_2$  production rates of the control muscles and in the initial rates of the muscles subjected to pressure. Several workers have referred to the variability of frogs or their tissues in connection with seasonal changes (10, 19, 20).

When averages of all of the experiments at each  $\text{O}_2$  pressure, except 15 atm., are considered, it is apparent that the increase in the rate of  $\text{CO}_2$  production, expressed as per cent of the initial rate, is similar in all cases but less well maintained at the higher pressures (fig. 3A). Muscles subjected to 8 atm.  $\text{O}_2$  continued to produce  $\text{CO}_2$  at their elevated rates for many hours. The rate fell

below the initial one in 2 muscles, at the ninth hour in one case and at the eighteenth hour in the other. Two other muscles, for which there were no initial rates, also showed late declines in  $\text{CO}_2$  production.

After the initial rise, the  $\text{CO}_2$  production decreased sooner at high  $\text{O}_2$  pressures than at low. The decrease occurred in all runs at 15, 21, and 35 atm.  $\text{O}_2$  without exception, and in all at 8 atm.  $\text{O}_2$  except four, which subsequent experience indicated were too short to show it.

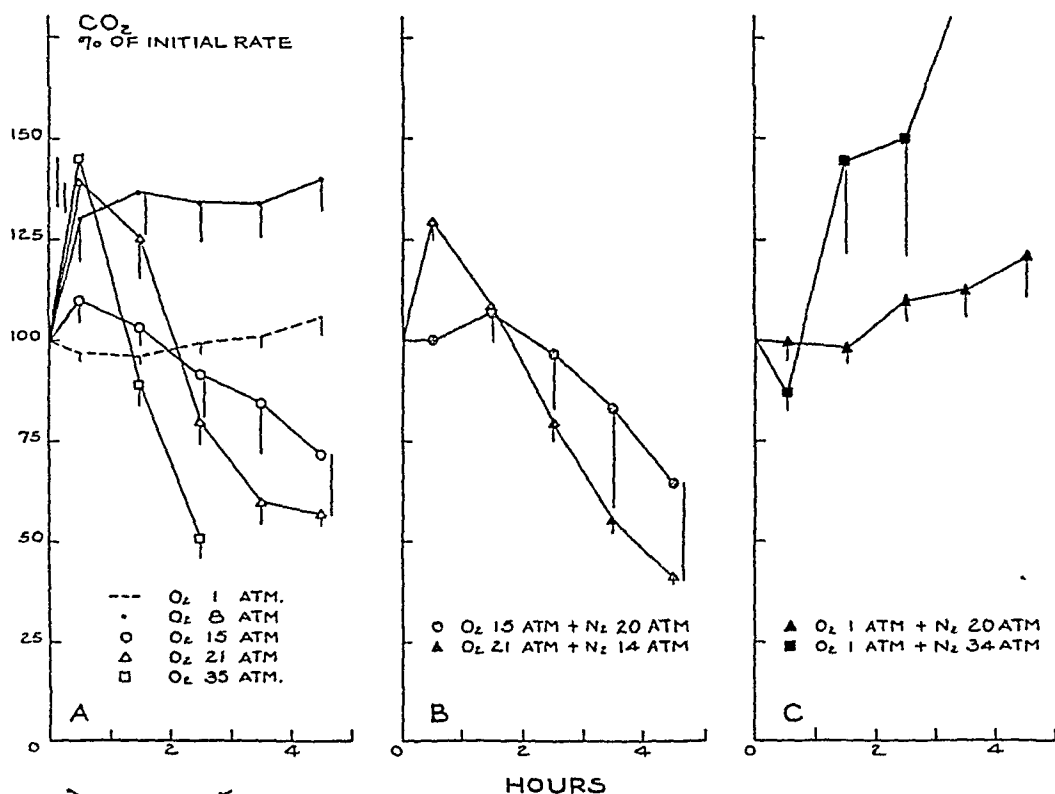


Fig. 3. Mean effects of various pressures of  $\text{O}_2$  and  $\text{N}_2$  upon the  $\text{CO}_2$  production of frog sartorii. The standard deviation of the mean is represented at each point by the vertical line. 0 hours indicates the beginning of compression. The numbers of experiments averaged together for each of these curves were (in order from first line of legend drawn) for A, 32, 6, 5, 12, 11; for B, 3, 3; and for C, 4, 2.

The lowest values of the  $\text{CO}_2$  production found at any pressures were between 6 per cent and 15 per cent of the initial rates. Each was 1 to 5 times the value of the correction used in calculating it. In experiments of sufficient duration to permit very low rates to be reached (fig. 4), the rates levelled off in a manner which suggests that there is a certain very small part of the muscle's  $\text{CO}_2$ -producing system which is unaffected by the inhibiting action of  $\text{O}_2$  at high pressures.

The general form of the family of curves (fig. 3A) suggests that increasing the  $\text{O}_2$  pressure exerts two influences which work simultaneously but in opposite directions: one, A, brings about a rapid increase in  $\text{CO}_2$  output without clear relationship to the pressure, in the range of  $\text{O}_2$  pressures employed; whereas another, B, causes a decrease in the  $\text{CO}_2$  output proportional to the  $\text{O}_2$  pressure.

B acts to depress  $\text{CO}_2$  production caused by A as well as that produced by the system functioning initially.

The attempt to distinguish between the action of high concentrations of  $\text{O}_2$  and the action of pressure *per se* upon the  $\text{CO}_2$  production of muscle led to a few experiments in which  $\text{N}_2$  was used to raise the pressure (fig. 3C). The  $\text{O}_2$  pressure remained at the control level of 1 atm. Four muscles under a total pressure of 21 atm. (1 atm.  $\text{O}_2$  + 20 atm.  $\text{N}_2$ ) showed little change for 2 hours but a slow increase in rate thereafter. At 35 atm. total pressure (1 atm.  $\text{O}_2$  + 34 atm.  $\text{N}_2$ ) a decrease in the mean rate (2 experiments) appeared during the first hour, but it was followed by a definite increase, which began in the second hour.<sup>3</sup> Of the 4 controls for  $\text{N}_2$  experiments, one, alone, showed a tendency to increase its  $\text{CO}_2$

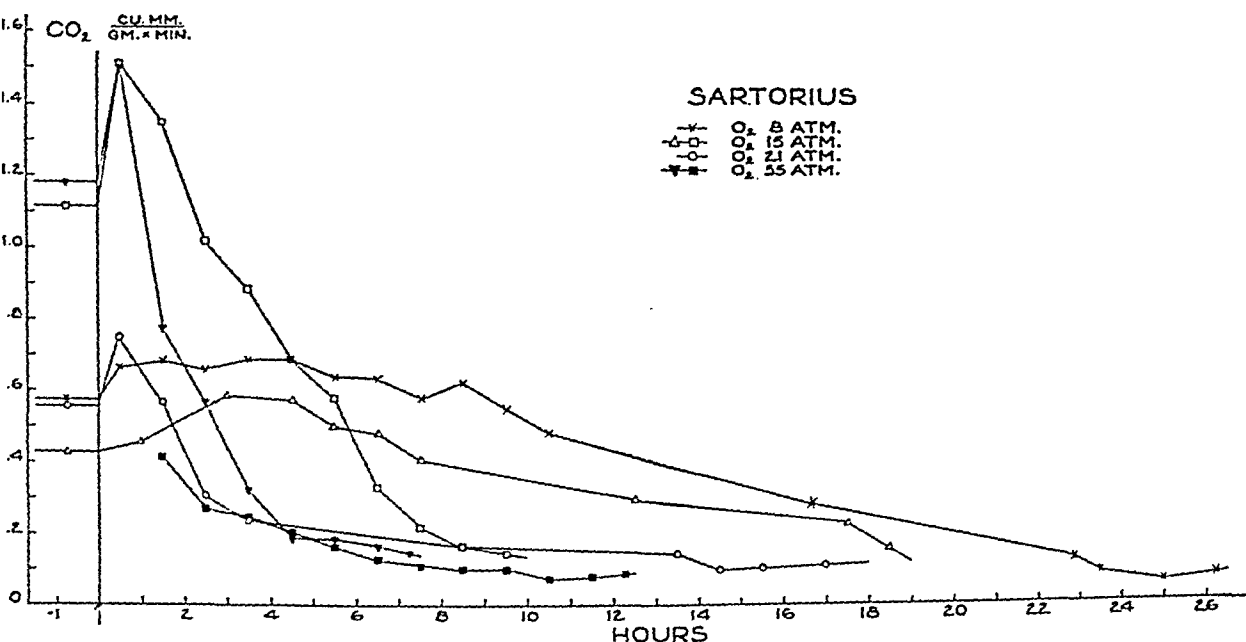


Fig. 4. Curves showing low rates of  $\text{CO}_2$  production of 6 individual frog sartorii under various high  $\text{O}_2$  tensions. (↑) indicates the beginning of compression. The resting rate for each muscle is shown by a straight line at the left of (↑). No resting rate was determined for the muscle represented by the solid square.

output with time; the others maintained their outputs or reduced them slightly. These experiments, employing  $\text{N}_2$  to augment the pressure, strongly recommend the conclusion that both the initial increase and the later decrease in  $\text{CO}_2$  production of muscles at high  $\text{O}_2$  pressures are due to the  $\text{O}_2$  tension rather than to the pressure involved. Moreover, the inhibition is even greater if muscles under 21 or 35 atm. total pressure (1 atm.  $\text{O}_2$  + 20 or 34 atm.  $\text{N}_2$ ) rather than those at 1 atm. total pressure (1 atm.  $\text{O}_2$ ) are considered controls for muscles under 21 or 35 atm.  $\text{O}_2$ .

It appears, therefore, that  $\text{N}_2$  at high pressure, unlike  $\text{O}_2$ , not only does not cause a decrease in  $\text{CO}_2$  production of isolated muscles but actually stimulates

<sup>3</sup> Two technically faulty experiments, omitted from the averages, also pointed toward an increase in  $\text{CO}_2$  output by muscles under 1 atm.  $\text{O}_2$  + 34 atm.  $\text{N}_2$ .

them to increased production. The work reported in this paper gives no clue as to whether the pressure or some specific action of the  $N_2$  on the tissue is responsible for the increased  $CO_2$  production. However, increased pressure (hundreds of atmospheres) will cause reversible contraction of muscle (21) together with chemical changes normally associated with contraction (22). Moreover, pressure, within limits, causes an immediate increase in the amplitude of contractions of cardiac muscle (23) and favors the development of tension in single contractions of striated muscle (24) of cold blooded vertebrates. Brown (25, 26) concluded that "the extra tension due to pressure is induced by pressure only during the initial eighth of the contraction phase. During this period pressure causes the mobilization of an extra amount of a contractile catalyst from

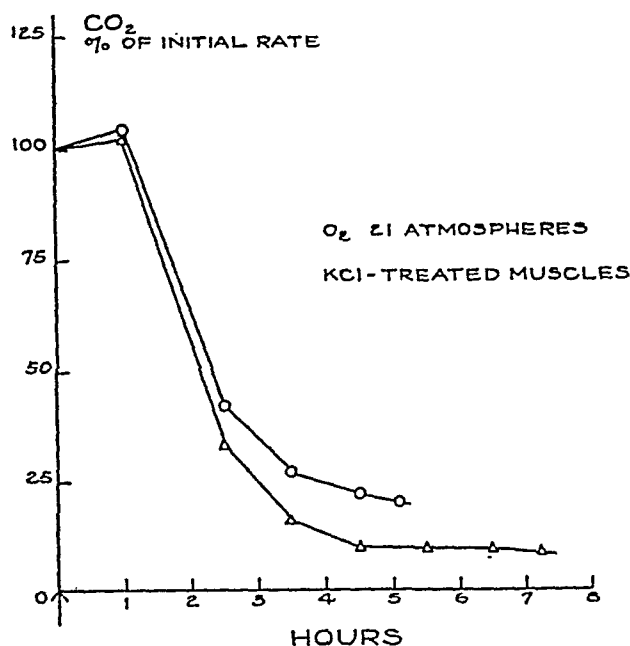


Fig. 5.  $CO_2$  production of 2 muscles with initial rates of  $CO_2$  output elevated by the action of KCl. ( $\uparrow$ ) indicates the beginning of compression.

its precursor". In view of the demonstrated effects of high pressures upon certain chemical reactions within muscle, it is not unreasonable to suspect that pressure, alone, may be responsible for some augmentation of the activity of the normal energy-yielding processes of muscle even at the 21 to 35 atm. used in this work.

Further evidence that the  $O_2$  concentration, and not the pressure, is responsible for the two responses of the  $CO_2$ -producing system of muscle to high  $O_2$  tension, is found in experiments under a total pressure of 35 atm., of which 15 or 21 atm. are  $O_2$  and the remainder  $N_2$  (fig. 3B). It is evident that the curves for the mean values of these  $O_2$ - $N_2$  experiments are more closely related to the curves for the corresponding  $O_2$  pressures (fig. 3A, 15 and 21 atm.  $O_2$ ) than to the curve for the corresponding total pressure (fig. 3C, 1 atm.  $O_2$  + 34 atm.  $N_2$ ).

Two muscles in which there were high initial  $CO_2$  production rates, due to stimulation by previous immersion of the muscles in isotonic KCl solution, diminished their outputs of  $CO_2$  while under 21 atm.  $O_2$  (fig. 5), whereas a control



maintained the high rate established initially. The fact that the extra  $\text{CO}_2$  production, stimulated by KCl, was affected, indicates that the portion of the muscle's respiratory system which functions during activity (20), as well as that which functions during rest, is sensitive to  $\text{O}_2$  at high pressure.

Muscle is not alone in its response to high  $\text{O}_2$  tensions. Figure 6 shows that other tissues are likewise sensitive and to a similar degree. However, certain differences appear among the curves in figure 6 and figure 3A. No increase in  $\text{CO}_2$  output occurs during the first hour in liver or skin. Liver and possibly lung show a tendency to level off somewhat above zero  $\text{CO}_2$  production, whereas kidney and skin do not. In nerve,  $\text{CO}_2$  production falls to 50 per cent of its initial rate more quickly than in any other tissue. Too few runs have been done to warrant more than a brief word about these observations. It is possible that increases do not appear in liver and skin records simply because the peaks were small and the readings were not sufficiently close together to catch them. Moreover, the liver controls show declines in  $\text{CO}_2$  output which would make it doubly hard to find transient rises. Two of the three curves for liver, at 35 atm.  $\text{O}_2$ , do show less precipitous drops for the first than for the second hour.

Unique experiments of two sorts gave suggestions concerning the state of the  $\text{O}_2$ -poisoned muscle while it was under high  $\text{O}_2$  pressure. When the preformed  $\text{CO}_2$  was determined for a muscle under 35 atm.  $\text{O}_2$  and for a control at 1 atm.  $\text{O}_2$ , the value for the former was only 11 per cent of that needed to explain the low  $\text{CO}_2$  output on the basis of the retention of  $\text{CO}_2$  by buffers within the muscle. Moreover, the fact that the preformed  $\text{CO}_2$  of the  $\text{O}_2$ -poisoned muscle was somewhat less than that of the control suggests that an accumulation of one or more acid metabolites may have driven off  $\text{CO}_2$ .

The very slight response to the pouring of isotonic KCl solution over an  $\text{O}_2$ -poisoned muscle, before decompression, indicated that such a muscle's ability to increase its  $\text{CO}_2$  output, in response to the action of KCl upon it, is greatly impaired.

Various attempts were made, after decompression, to get information concerning the state of the  $\text{O}_2$ -poisoned muscle. In many cases, the muscle's ability to twitch, in response to mechanical or electrical stimulation, was tested after the muscle was removed from the conductivity cell.

Of the muscles exposed to  $\text{O}_2$  under pressure for periods ranging up to 16 hours in length, 10 of 37 tested, or 27 per cent, responded, whereas 38 of 48 tested, or 79 per cent, of the controls and other muscles at atmospheric pressure, tested within 23 hours of the beginning of the experiment, responded. Only one muscle exposed to  $\text{O}_2$  at 35 atm. twitched afterward and that muscle had been subjected to high  $\text{O}_2$  pressure for only  $2\frac{1}{4}$  hours. Several later experiments, at the same pressure, were stopped at the end of 3 hours but in none did the muscle respond to a mechanical stimulus. It is noteworthy, also, that none of the muscles exposed to 8 atm. or 15 atm.  $\text{O}_2$  which twitched at the end of the experiment had dropped to a low rate of  $\text{CO}_2$  production. Except for the one muscle at 35 atm.  $\text{O}_2$ , only at 21 atm.  $\text{O}_2$  did muscles reach low rates yet twitch when removed from the cell. Several of them survived 7 hours of  $\text{O}_2$  at high pressure plus about 5 additional hours before and after the pressure period. It becomes apparent that loss of the

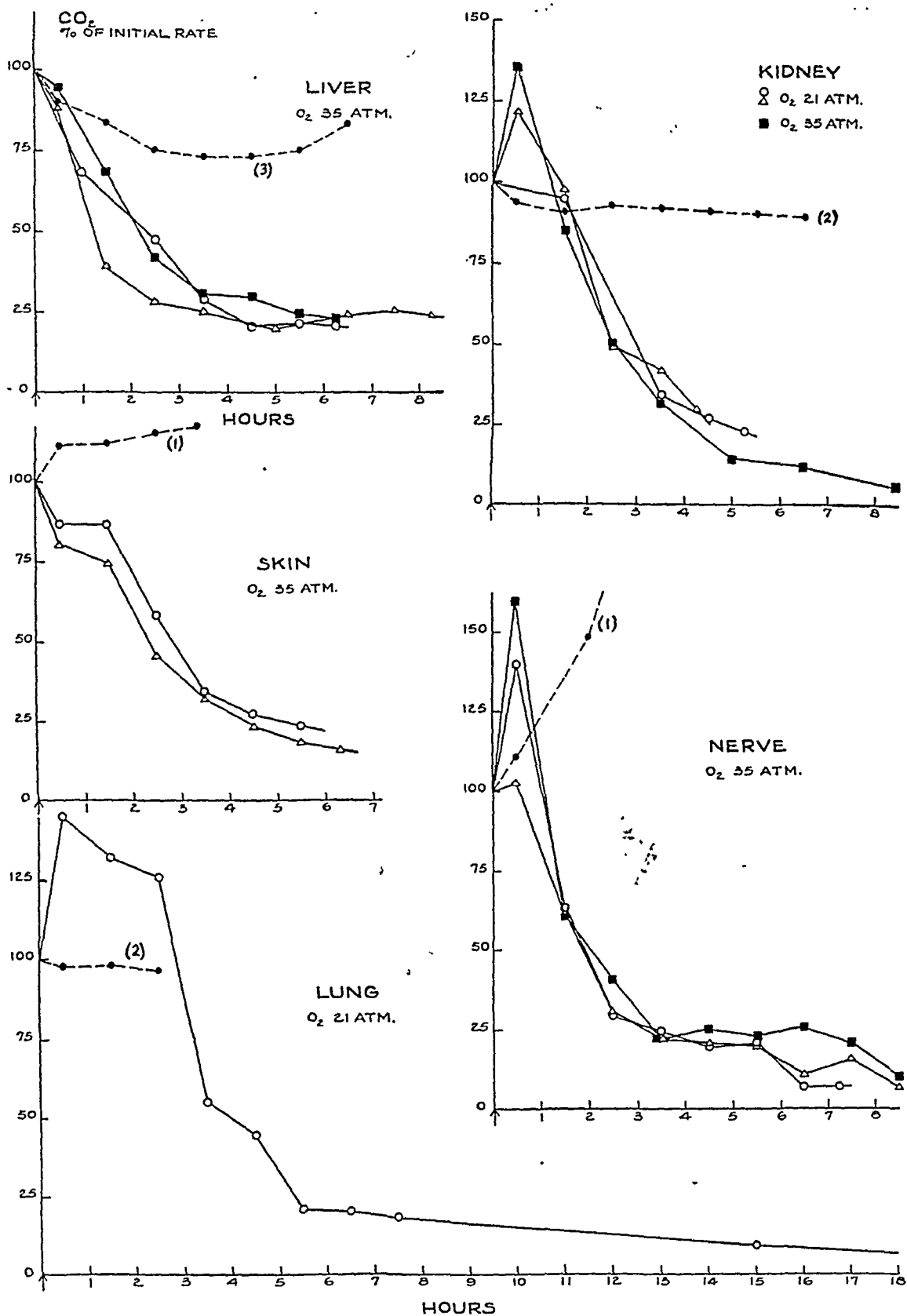


Fig. 6.  $\text{CO}_2$  production of liver, kidney, skin, nerve, and lung under 21 or 35 atm.  $\text{O}_2$ . (↑) indicates the beginning of compression. Broken lines indicate mean of control experiments at 1 atm.  $\text{O}_2$ . The number of experiments is indicated by figures in parentheses. Otherwise each curve represents a single experiment.

ability to contract is one result of  $O_2$  poisoning and that the higher the  $O_2$  pressure the shorter the exposure necessary to bring about this change.

The curves for  $CO_2$  production of muscles at 21 atm.  $O_2$  (fig. 3A) appeared to be approaching an asymptote considerably higher than that of curves for muscles at other  $O_2$  pressures. This suggests that in these muscles a relatively large fraction of the  $CO_2$ -producing system was uninjured, or only slowly poisoned by  $O_2$  at high pressure. All of these muscles were run during July–December 1941, a period in which none of 15 muscles run at atmospheric pressure lost its ability to contract. It was also the time during which frogs, judged by the low resting rates of their muscles, were in very good condition. These observations, plus the previously mentioned slowness of these muscles to lose their ability to contract, point toward a possible relationship between resistance to  $O_2$  poisoning and “good condition” of a muscle.

Characteristically the  $O_2$ -poisoned muscle shortened and became white, opaque and stiff. All degrees of these changes were observed. Almost all of the muscles observed after 3 hours at 35 atm.  $O_2$  were beginning at least to shorten and to look opaque in spots. Some muscles were shortened to about  $\frac{1}{3}$  of their original length and had a coagulated appearance. Muscles which were able to twitch looked normal or, perhaps, slightly wrinkled. Control muscles commonly looked normal for as long as 12 hours and for  $16\frac{1}{2}$  to 23 hours, in three cases. It is instructive to compare the changes in the muscle run at 8 atm.  $O_2$  for 28 hours with the changes in its control muscle observed after 25 hours. Although the  $CO_2$  production of the former never fell much after climbing to 180 per cent of its resting rate, the muscle was rather opaque. The control, on the other hand, was dried, yellowish, and very translucent. Neither was shortened.

That the ability to produce  $CO_2$  reappears in  $O_2$ -poisoned muscles when they are returned to atmospheric pressure is demonstrated by the sharp rise in almost all of the curves at decompression (indicated by  $\downarrow$ , fig. 7). There is a conspicuous overshooting of the initial rate (100 per cent) and a late downward trend. It is improbable that the increases following release of pressure can have been due to spatter of the  $Ba(OH)_2$  solution because the muscles, except in the earliest experiments, were protected by a glass cloth shield between them and the solution in the bottom of the cell. Recovery, judged by the  $CO_2$  production rates during the second hour after decompression (when both the muscle and the  $Ba(OH)_2$  solution have had the opportunity to approach a new equilibrium), appears to be slower the longer the exposure to the high  $O_2$  tension. Of the two muscles which were slowest to begin their recovery, one had been under 21 atm.  $O_2$  for 8 hours and 20 minutes and the other (not shown in the graph) under  $O_2$  at a pressure which rose from 15 to 24 atm. during 6 hours and 50 minutes. These results support the idea that high  $O_2$  tension brings about an irreversible or slowly reversible change in the  $CO_2$ -producing systems of the muscle, and that the extent of the damage is related to the time over which the  $O_2$  at high pressure acts.

Recompression, after the  $CO_2$  production rate had returned to a high level, caused a second reduction in  $CO_2$  output (fig. 8). In each of the two muscles so treated,

the rate of  $\text{CO}_2$  output was falling at the time of the second compression. Although neither muscle exhibited the increase in rate characteristic of first com-

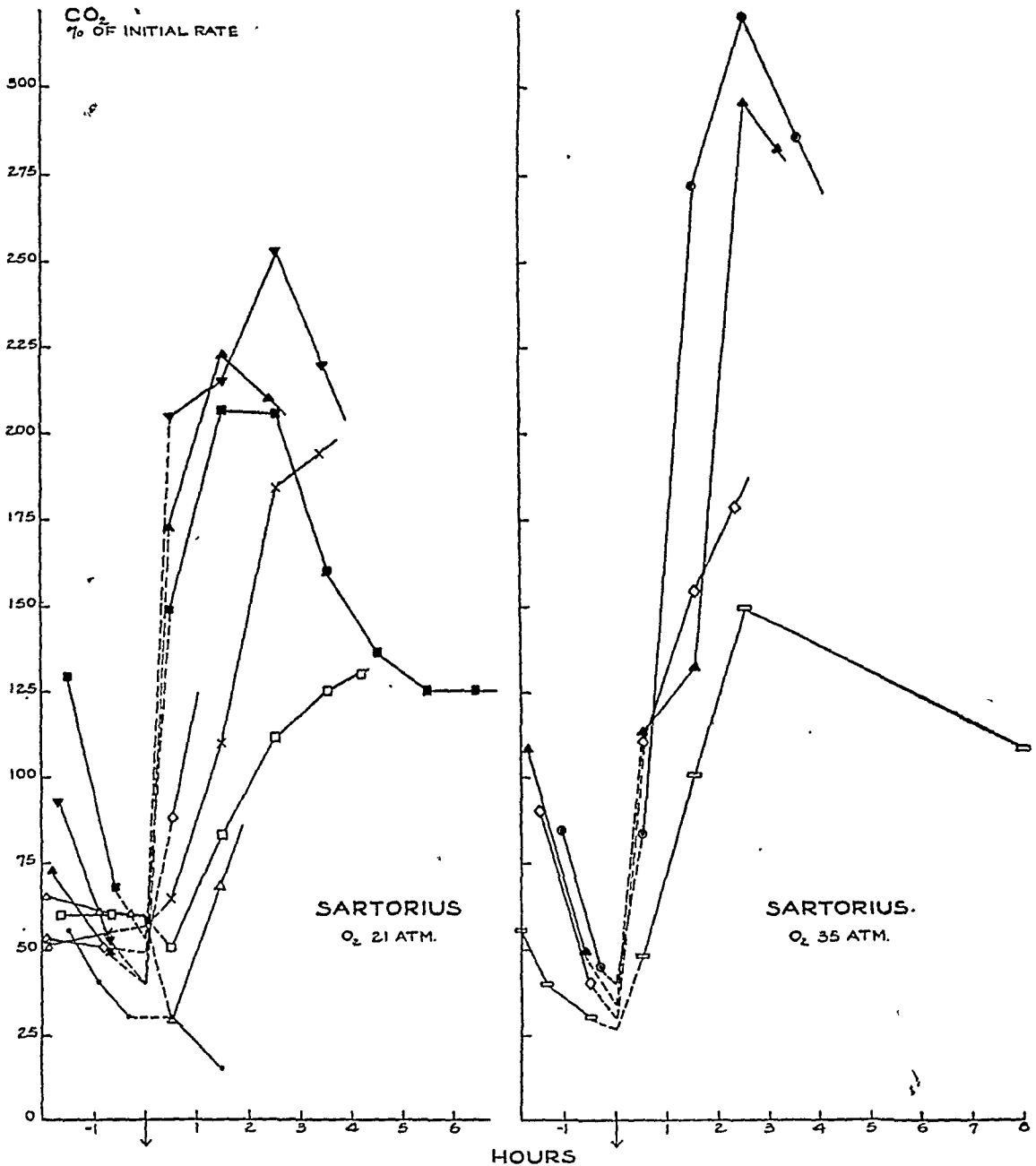


Fig. 7.  $\text{CO}_2$  production of muscles decompressed beginning at ( $\downarrow$ ). In each case a broken line extends the curve along a course suggested by the preceding points and then runs to the first point determined after decompression.

pressions, one (21 atm.  $\text{O}_2$ ) did show a slight check in the decline of the rate during the first hour. The  $\text{CO}_2$  production rate of this same muscle levelled off and remained for the last 100 minutes of the experiment at 0.35 cu. mm./gram/min., slightly below its original resting rate of 0.40 cu. mm./gram/min. The rate

of the other muscle was continuing to fall when the experiment was terminated but had already reached 50 per cent of its initial rate.

The fact that upon decompression the  $\text{CO}_2$  production returned to its initial level, or to a point above it, indicates that the  $\text{CO}_2$  production had diminished because of the presence of the high  $\text{O}_2$  tension and not because of some factor such as the depletion of glycogen stores, or because of the development of rigor. Further evidence to support this view lies in the fact that control muscles, with two

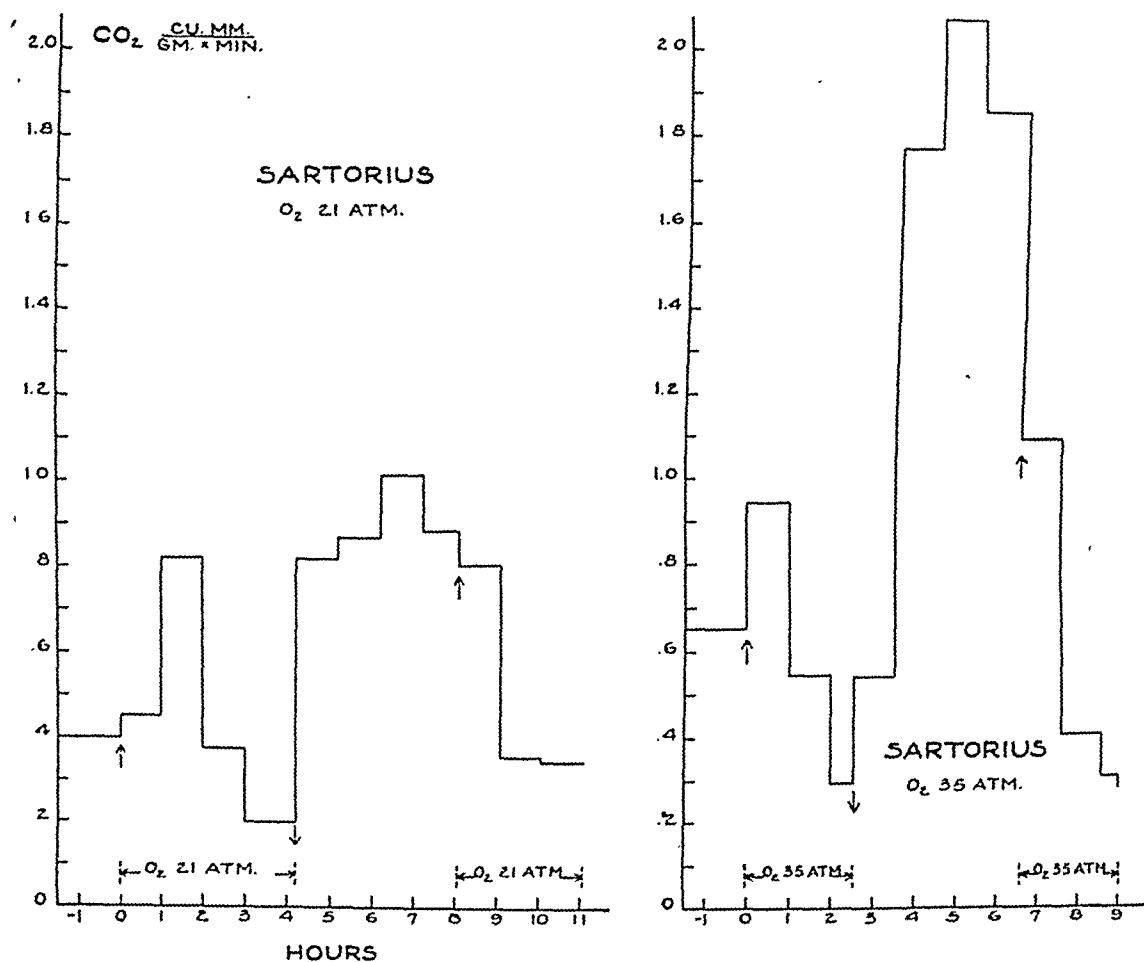


Fig. 8.  $\text{CO}_2$  production of 2 muscles, one at 21 atm.  $\text{O}_2$ , the other at 35 atm.  $\text{O}_2$ . Compression begins at ( $\uparrow$ ), decompression at ( $\downarrow$ ). In each case a second compression occurred.

doubtful exceptions, maintained a normal  $\text{CO}_2$  production rate or increased it, sometimes to a very high rate in the longest runs. Finally, in one muscle, exposed to 1 atm.  $\text{O}_2$  plus 20 atm.  $\text{N}_2$ , the rate of  $\text{CO}_2$  output rose over a period of 20 hours and remained unchanged following decompression.

DISCUSSION. The reduction in  $\text{O}_2$  consumption and  $\text{CO}_2$  production in  $\text{O}_2$ -poisoned animals, first observed by Bert (1), points to a decreased tissue metabolism. The idea that such a decrease could be attributed to an inhibition of oxidative enzymes was much later supported by the demonstration of the inactivation *in vitro* of certain enzymes or enzyme systems in the presence of  $\text{O}_2$  at high pressure (27-33). Bean (34) and Stadie, Riggs and Haugaard (35) have

reviewed the literature and have pointed out ways in which  $O_2$  may act to inhibit enzyme activity. Recently Stadie et al. (16, 17) have reported a gradual decline in the  $O_2$  consumption of rat tissues exposed to high  $O_2$  pressures comparable to the diminution in  $CO_2$  output of frog tissues similarly treated (15). It is understandable that the frog tissues, run at  $22^\circ C.$ , were slower than the rat tissues, run at  $38^\circ C.$ , to show the depressing effect of a high concentration of  $O_2$  upon their metabolism.

The increase in  $CO_2$  production which follows the application of high  $O_2$  pressure deserves consideration even though it cannot be explained on the basis of the work presented here. Its persistence for hours in muscles under 8 atm.  $O_2$ , its absence in liver and skin, in acid agar plus  $CaCO_3$ , and in muscles following a second compression with  $O_2$ , and its insignificance in muscles stimulated with KCl, discredit the possibility that it is due to inaccurate correction of the data for the small amount of  $CO_2$  in the  $O_2$  used to increase the pressure.

The continuance of the high rate of  $CO_2$  output at 8 atm.  $O_2$  suggests that the central part of a sartorius muscle may be hypoxic at 1.0 atm.  $O_2$ , but may be adequately supplied at  $O_2$  pressures of 8 atm. or more. One would expect, then, lower initial rates and greater increases in rates of  $CO_2$  production, after compression with  $O_2$ , in the large muscles than in the small ones. Efforts to establish such correlations within the group of eleven muscles exposed to 35 atm.  $O_2$  were unsuccessful. However, the possibility remains that in some of the muscles with the highest rates of  $CO_2$  output in the first hour after compression, improved diffusion of  $O_2$  to the center of the tissue may have contributed to the rise in  $CO_2$  output after compression.

There is the possibility that pressure, alone, stimulates a  $CO_2$ -producing system of muscle under high  $O_2$ . Reference has been made already, in the presentation of results, to the accumulating evidence in support of the idea that pressure favors certain steps in the controlled release of potential energy of muscle. However, in the few experiments of this study in which the pressure was increased by  $N_2$ , the increase in  $CO_2$  output was regularly later in appearing than in runs in which the pressure was increased by  $O_2$ . Consequently (if the whole action of  $N_2$  at high pressure is attributed to the pressure and not to the increased concentration of  $N_2$ ), it is unlikely that pressure *per se* is responsible for the sharp increase in  $CO_2$  production found within the first hour after compression with  $O_2$ . Pressure could, nevertheless, be related to the increased rate maintained by some muscles at 8 atm.  $O_2$ .

It may be that some oxidative reactions within a tissue are accelerated by high  $O_2$  concentration even though others are inhibited. Stadie, Riggs and Haugaard (17) have been unable to demonstrate a rise in  $O_2$  consumption in rat tissues after compression with  $O_2$ , and feel that "if such occurs, it must be less than 10 to 15 per cent of the total oxygen uptake".

The accumulation of acid metabolites within a tissue suspended in gas would result in a loss of  $CO_2$  from its buffers. A single muscle tested for preformed  $CO_2$  at the end of 6 hours of exposure to  $O_2$  at 35 atm. did have less  $CO_2$  per gram of tissue than the control muscle but the experiment gave no clue to the time at

which the deficit developed. One other study (16) has also presented evidence to suggest that a high  $O_2$  pressure increases the rate of glycolysis.

The close correspondence between the effects of  $O_2$  at high pressure upon the contractile power (10) and  $CO_2$  production of frog sartorius muscles is very striking. Contraction height measurements were made at about 5 atm.  $O_2$  and the most nearly comparable  $CO_2$  measurements at 8 atm.  $O_2$ . Each showed an initial increase, followed by a decrease, recovery upon decompression (if the compression was not too much prolonged), and less delay in the appearance of the decrease after a second compression. However, conspicuous differences exist in the time of appearance and the course of the observed decreases.

No sign of diminution in  $CO_2$  production appeared until the third hour under high  $O_2$  tension whereas decrease in contraction height occurred as early as  $1\frac{1}{2}$  hours after compression with  $O_2$ .<sup>4</sup> Furthermore, the drop in  $CO_2$  output was very gradual and prolonged, not reaching the precompression rate for 9 to 18 hours in 2 muscles which showed it. In 2 other muscles, for which no determination of initial rate was made, the decrease obviously came late. The fall in contraction height on the other hand, was rapid, reaching about  $\frac{1}{3}$  of the high value due to compression within 2 hours after the decline set in.

Thus, from the foregoing comparison, it appears that some necessary step related to the contractile mechanism is inhibited more rapidly than the compound or system, the inactivation of which brings about a decrease in  $CO_2$  output. This is borne out by the observation in this study that many muscles were unable to twitch at the termination of the experiment, although their production of  $CO_2$  had risen sharply, following decompression.

The way in which  $O_2$  at high tension induces the restlessness and convulsions, characteristic of acute  $O_2$  poisoning, is not yet clear. Inasmuch as these symptoms are evidently of central nervous system origin, it is noteworthy that nervous tissue, judged by decreases in its  $O_2$  consumption (16, 17) and in its  $CO_2$  production (15), is more susceptible than any other tissue to poisoning by  $O_2$ . The fact that the symptoms of acute  $O_2$  poisoning, even death, appear in rats sooner after compression than do decreases in the  $O_2$  consumption of rat brain slices (16) does not rule out the possibility that some metabolic change, perhaps not significant in the over-all  $O_2$  consumption of an isolated tissue, may be responsible for early derangements in central nervous system activity. Two signs of such early responses to high  $O_2$  concentrations are to be found (1) in the increase in the  $CO_2$  production of some tissues before the inevitable decrease appears, and (2) in the irreversible change which occurs in rat brain slices causing their  $O_2$  consumption to fall even though they are decompressed before the decline in  $O_2$  uptake appears (16).

#### SUMMARY

1. High  $O_2$  pressure (8–35 atm.) causes a decrease in the  $CO_2$  output of isolated frog sartorii; the decrease occurs sooner the higher the  $O_2$  pressure employed.

<sup>4</sup> These are minimum times; the average times for the appearance of both these effects were considerably longer.

2. That the  $O_2$ , and not the pressure, is responsible for the decrease is evident from the following two facts: Increasing the pressure with  $N_2$  caused no decrease in  $CO_2$  production. When  $O_2$ - $N_2$  mixtures were used, the decrease in  $CO_2$  production was related to the partial pressure of  $O_2$  rather than to the total pressure.

3. Upon acidification, an  $O_2$ -poisoned muscle freed an amount of  $CO_2$  equal to only 11 per cent of the deficit in  $CO_2$  output which had been incurred by the muscle during its subjection to high  $O_2$  pressure. This indicates that the lowering of the  $CO_2$  output of a muscle in the presence of a high  $O_2$  tension cannot be attributed to the retention of  $CO_2$  by buffers in the muscle. In addition, the accumulation of acid metabolites in an  $O_2$ -poisoned muscle is suggested by the fact that the preformed  $CO_2$  per gram of this muscle was somewhat lower than that of the control muscle.

4. "Activity"  $CO_2$  production induced by KCl is inhibited by high  $O_2$  pressure.

5. Loss of the ability to twitch precedes alterations in the appearance of the  $O_2$ -poisoned muscles. Each of these changes is a function of the product of  $O_2$  tension and time.

6. That the change in the  $CO_2$ -producing system is reversible, at least, in part, is indicated by the fact that following decompression,  $CO_2$  production rises to a point above the initial rate.

7. The fact that the second compression, in two instances, caused a quicker decrease in  $CO_2$  output than the first compression, points to the existence of a cumulative effect in the action of high  $O_2$  pressure upon some  $CO_2$ -producing system of muscle.

8. High  $O_2$  pressure appears to cause an increase in  $CO_2$  production immediately after compression.

9. Kidney, liver, skin, lung and nerve also respond to high  $O_2$  pressure by increased  $CO_2$  production. The rate of  $CO_2$  output falls to 50 per cent of the initial rate more quickly in nerve than in any other tissue. Early increase in rate appeared only in kidney, lung and nerve.

10. Apparatus is described which makes possible the measurement of the  $CO_2$  production of a tissue during its exposure to high pressure of  $O_2$ .

The writer is deeply indebted to Prof. W. O. Fenn for his counsel and encouragement.

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# SOME EFFECTS OF d-TUBOCURARINE ON OXIDATIONS IN MAMMALIAN TISSUES<sup>1</sup>

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A few preliminary studies made in this laboratory on minced curarized rat muscles indicated some striking changes from normal in the oxidative reactions of muscle. Although many of the physiologic actions of curare alkaloids have been described and clinical investigations with purified curare extracts have been carried out on a broad scale (1), the mechanism of action remains poorly understood. It was deemed desirable in view of the present wide use of the purified curare extracts in anesthesia, in convulsive shock therapy, and in many other instances, to learn the extent of the effect of the purified compounds on the metabolic reactions in muscles and brain. Gross disturbances of this nature, if present, might delay the return of normal tissue function. Dubinskii and Kuznetsov (2) showed in 1935, through oxygen and carbon dioxide studies on blood drawn from the femoral arteries and veins of cats, that the gaseous exchanges of muscle were decreased by concentrations of curare which did not paralyze respiration. However, the oxygen consumption of frogs and toads was found to have been increased 27 per cent by curarization (3) and the dehydrogenating capacity of tissues measured by reduction of dinitrobenzene was reported to have been increased following the administration of curare (4). Apparently no other attempts to study the effects of curare on oxidations in muscle or brain have been reported. The availability to us of ample quantities of pure d-tubocurarine chloride<sup>2</sup> made possible an attempt to study more directly the effects of the purified curare alkaloid on oxidative reactions in muscle and brain.

**EXPERIMENTAL.** Standard manometric techniques were used to obtain oxygen consumption data on strips of the sternohyoid muscle of the dog and rat, pieces of diaphragm of the rat, minces of the sternohyoid muscle of the dog, gastrocnemius muscle of the rat and guinea pig, and hashed brain of the rat. Tissues from 110 rats, 8 guinea pigs, and 16 dogs were used in these experiments.

*Preparation of tissues.* Those tissues obtained from rats and guinea pigs without anesthesia were removed and chilled rapidly after killing the animal by a sharp blow (except in brain experiments) and severance of the head. Minces were prepared using the mincer described by Seevers and Shideman (5); diaphragms were cut into strips; rat sternohyoid muscles were separated with needles into strips of suitable diameter; and brain tissue was hashed with a knife. Anoxia was prevented in the animals given d-tubocurarine by manually maintaining respiration with oxygen. In some cases rat gastrocnemii were removed under

<sup>1</sup> Presented before the Section on Anesthesiology, American Medical Association, in San Francisco, July 4, 1946.

<sup>2</sup> Provided by E. R. Squibb and Sons.

cyclopropane anesthesia, muscle being taken from one leg before and from the other leg after the injection of d-tubocurarine intraperitoneally.

Cyclopropane without premedication was the anesthetic chosen for the dog experiments. Anesthesia was induced in dogs by the carbon dioxide absorption method, using a 50-50 cyclopropane-oxygen mixture to bring the animals to the second plane, third stage. The animals were then intubated by direct vision orally and first plane anesthesia was maintained until the curarized strip was clamped off. Immediately following the intubation the animals were prepared for the aseptic removal of the sternohyoid muscle. The muscle was easily separated into two large groups of fibers. The bundle on the right side was then clamped off, and a long strip of the muscle weighing 3-5 grams (approximately one-half of the bundle) was removed and placed in cold Ringer-phosphate solution. The d-tubocurarine solution was then injected into one of the tongue veins and after a period of three minutes, during which time the respiration was necessarily maintained manually, the bundle of fibers on the left side was clamped off and another strip of muscle of equal size was excised and placed in cold buffer solution. The dogs were then sutured and suffered no apparent serious loss of function from the procedure.

Immediately following the removal of the second piece of muscle, the two samples were separated alternately into long bundles of fibers (6). Two or three strips measuring 0.3-0.5 mm. in thickness and weighing a total of approximately 250 mgm. were blotted with filter paper, weighed on the microtorsion balance, and placed in the flasks. Quadruplicate determinations from control and curarized strips were made in all cases. In those instances where a mince of the dog sternohyoid muscle was used, the procedure was the same as outlined above, except that the stripping technic did not precede the mincing. One experiment was conducted with dog strips removed at different times during cyclopropane-anesthesia and no curare alkaloids were used.

*Metabolism studies.* Standard Warburg manometric techniques were employed to determine the cubic millimeters of oxygen consumed per milligram wet weight of tissue per hour, which is given the usual designation of  $Q_{O_2}$ . Calcium ions were omitted routinely from the usual Krebs-Ringer-phosphate (7) solution for all mince experiments. The substrate was lithium pyruvate for muscle experiments and glucose for brain experiments. Oxygen was employed as the gas phase and the measurements were made at 38°C.

Time was found to be a major factor in the preparation of tissues only when minces were used. A standard interval of 26 minutes between the time of killing the animal and the first reading was maintained for the rat tissues and other minces. The time required to prepare strips of dog muscle and equilibrate the vessels was 50-60 minutes in all cases. Rat brains were prepared for readings in 40 minutes.

Pyruvate utilization was determined in a few instances by measuring, by the Friedemann-Haugen method (8), the amount of substrate remaining in trichloroacetic acid extracts of the flask contents following the experimental period. The dose of d-tubocurarine chloride given each group of animals was determined ex-

perimentally. Criteria of adequate dosage in rats and guinea pigs were inability to pull up the hind legs and embarrassed respiration within a 5 minute period; in anesthetized dogs, the failure of automatic respiration. The importance of not using paralysis of the diaphragm as the sole criterion in rats is emphasized by West (9).

The numerical-values cited are given as the means or the means and standard errors. Fisher's *t* values above 2.0 indicate a significant difference between the means of control and experimental groups of measurements.

RESULTS. Preliminary determinations of the oxygen consumption of 20 rat and 8 guinea pig gastrocnemius muscle minces indicated that the injection of d-tubocurarine increased in some way the tempo of oxidative reactions in muscle to the extent of 40-100 per cent, regardless of the presence or absence of 0.01 M pyruvate or succinate as substrate. Calcium ions were found to decrease the oxygen consumption of gastrocnemius minces by about 85 per cent, and, since the curarized muscle minces respired more rapidly with or without calcium in the media, these ions were omitted routinely from the buffer solutions used in mince, but not in other, experiments in order to continue working with relatively high rates of oxygen consumption.

Pertinent data on the oxygen consumption of the various tissues studied are recorded in table 1. Figure 1 illustrates the type of curve obtained in the mince experiments and in figure 2 the typical straight line curve obtainable with the more intact tissues such as diaphragm and sternohyoid muscle strips is shown. Cyclopropane anesthesia *per se* was shown to have no effect on the  $Q_0$ , for muscle strips from the dog in an experiment simulating those in which d-tubocurarine was injected. When the oxygen consumption curves for minced gastrocnemius muscles from the right legs of 4 rats anesthetized with cyclopropane were compared with those of minces of the left gastrocnemii removed 6 minutes later following, in 2 cases, the injection of d-tubocurarine, it was shown that cyclopropane over a period of time had no apparent effect on the rate of oxidations, and that the increase illustrated in figure 1 as being due to d-tubocurarine occurred regardless of the anesthesia. Pyruvate determinations on the flask contents of the dog sternohyoid mince experiment (dog 12, table 1) indicated that the curarized mince had used 6.9  $\gamma$  pyruvate/mgm. tissue as opposed to 5.4 for the control mince. The *in vitro* addition of d-tubocurarine to minced sternohyoid muscle from another dog resulted in a mean value of 2.480 cmm. oxygen/mgm. mince in 2 hours as opposed to a control mean of 2.220. Muscle removed from the same dog following the injection of d-tubocurarine was found to consume 2.600 cmm. of oxygen/mgm. mince in 2 hours. Although the data are not shown in table 1, the rate of respiration for the muscle strip, diaphragm, and brain preparations was found to be nearly constant for more than two hours. Data from other dog experiments indicated that the variations among dogs might have been due partially to slight variations in the level of anesthesia and emphasized the necessity of alternate stripping of the curarized and control tissues during the preparation of the muscle strips for the reaction vessels.

DISCUSSION. The data cited from the preliminary experiments on rat and

guinea-pig gastrocnemius minces were considered indicative of a positive effect of d-tubocurarine on the metabolism of muscle minces of these animals. Pyruvate was chosen over succinate for the major portion of the experiments because py-

TABLE 1

*Effect of d-tubocurarine on the oxygen consumption of tissues; 0.01 M Pyruvate was used as substrate unless otherwise noted*

ANIMAL	TISSUE PREPARATION	d-TUBO-CURARINE*	Q <sub>O<sub>2</sub></sub>		DIFFERENCE	
			Control	Curarized	%	Fisher's t
Rat	Gastrocnemius muscle mince	In vivo	0.685 ±0.07(12)†	1.143 ±0.11(12)†	+67.0	3.500
Rat	Gastrocnemius muscle mince	In vitro	0.552 ±0.051(8)	0.620 ±0.058(8)	+12.3	0.884
Rat	Diaphragm pieces	In vivo	1.767 ±0.061(8)	1.650 ±0.052(8)	-6.6	1.427
Rat	Sternohyoid muscle strips	In vivo	0.727 ±0.025(4)	0.667 ±0.025(4)	-8.2	1.690
Rat	Brain hash	In vivo	2.126 ±0.173(9)	2.150 ±0.08(9)	+1.1	0.384
Dog, no. 1	Sternohyoid muscle strips	In vivo	0.697	0.695	-0.3	
2	Sternohyoid muscle strips	In vivo	0.699	0.693	-0.8	
3‡	Sternohyoid muscle strips	In vivo	0.331	0.338	+2.2	
4‡	Sternohyoid muscle strips	In vivo	0.365	0.381	+4.1	
5§	Sternohyoid muscle strips	In vivo	0.657	0.670	+2.0	
9	Sternohyoid muscle strips	In vitro	0.637	0.642	+0.8	
6	Sternohyoid muscle strips	In vivo & in vitro	0.661	0.670	+1.3	
12	Sternohyoid muscle mince	In vivo	(cmm. in 1.800 2 hours)	(cmm. in 2.350 2 hours)	+30.0	

\* Rats given 3 units/kgm. I.P.; dogs, 1.5 units I.V.; 2 units/flask used in *in vitro* studies.

† Figures in parentheses indicate number of animals used.

‡ No substrate used.

§ No calcium ions in the media.

|| Addition of 1 unit/flask and 0.33 unit/cc. to preparation bath of curarized tissue only.

ruvate seems to be more directly concerned than succinate in the bulk of oxidative reactions in muscle.

Data in figure 1 show clearly that the increase in oxygen consumption of rat gastrocnemius minces due to the injection of d-tubocurarine persists when fairly large groups of animals are used. The increase of 67 per cent and the Fisher's t value of 3.50 indicate that the difference is highly significant. The limited num-

ber of pyruvate studies indicates that the increase in oxygen consumption is paralleled by a greater utilization of pyruvic acid, although the relationship is not quantitative. It was considered important to learn whether or not this increase could be obtained simply by adding the d-tubocurarine to the reaction vessels. The *in vitro* data presented in table 1 show that such an increase is questionable. Although the average increase of 12.3 per cent in oxygen consumption of d-tubocurarine-added mince is derived from individual increases of 4-29 per cent, the variability of the  $Q_{O_2}$  values, reflected in the  $t$  value of 0.884

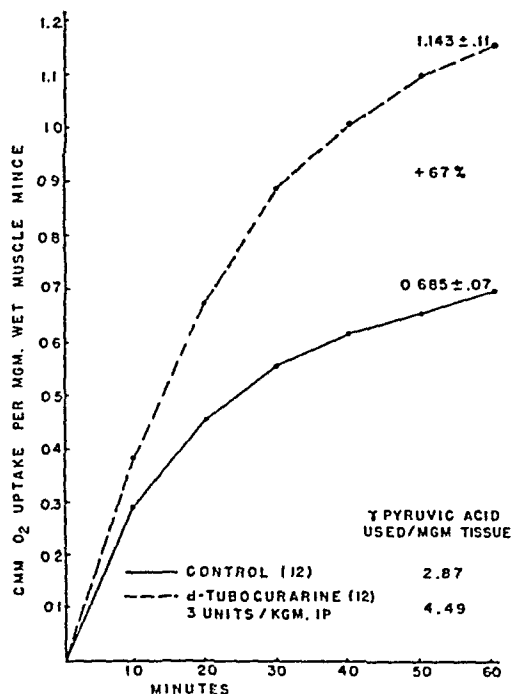


Fig. 1

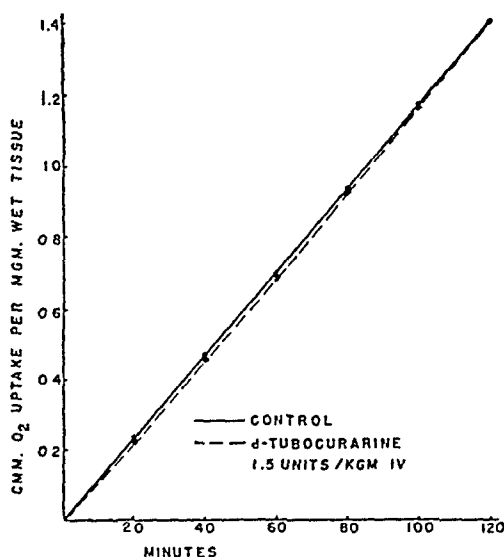


Fig. 2

Fig. 1. Effect of d-tubocurarine *in vivo* on oxygen consumption and pyruvate utilization of minced rat gastrocnemius muscle; 0.01 M Pyruvate was used as substrate. Pyruvate values cited are on 4 rats only with approximately average  $Q_{O_2}$  values. Fisher's  $t$  for oxygen data = 3.50.

Fig. 2. Effect of d-tubocurarine *in vivo* on oxygen consumption of dog sternohyoid muscle strips; 0.01 M Pyruvate was used as substrate.

casts considerable doubt on the significance of this difference when the group is considered as a whole.

The mincing of tissues is extremely destructive and the abnormal chemical processes set up in the presence of oxygen permit only brief survival (10). This is demonstrated well in figure 1, where it can be seen that approximately 50 per cent of the total oxygen used by control minces during the one hour period had been consumed by the end of 15 minutes. In an effort to test the effect of d-tubocurarine on muscle preparations which survive for longer periods, the dog sternohyoid strip technique (6, 10) was used. Figure 2 represents the typical constant rate of oxidative reactions obtainable with the more intact muscle preparations such as strips and diaphragm pieces. Oxygen consumption data

on strips of dog sternohyoid muscles removed under cyclopropane anesthesia before and after the intravenous injection of d-tubocurarine (table 1) showed no significant difference between curarized and control tissue. For none of the muscle strips from 7 dogs was this difference in  $Q_{O_2}$  greater than 5 per cent (fig. 2; table 1).

These determinations were made with pyruvate as substrate and in the presence of calcium ions. The evidence indicates that d-tubocurarine has no effect on oxygen consumption of these strips regardless of the presence of substrate or calcium ions, and regardless of the level of cyclopropane anesthesia, although the lack of substrate and the lower level of anesthesia caused decreases in oxygen consumption of both curarized and control strips. The addition, *in vitro*, of d-tubocurarine to strips was also ineffective in producing any increase over the control  $Q_{O_2}$  (table 1, dog 9). The data for strips from dog 6 show, as expected, that the absence of increased oxygen consumption in curarized tissue was not due to a washing out of the alkaloid during preparation of the strips.

Although cyclopropane anesthesia *per se* was shown not to be a factor in the constancy of the  $Q_{O_2}$  measurements on dog muscle strips, it was deemed desirable to learn if the anesthetic could be a factor which inhibited the d-tubocurarine effect noted on rat leg muscle minces. It has been clearly demonstrated through the use of anesthetized rats that such an inhibition does not occur.

Next the possibility of a species difference in the response to d-tubocurarine *in vivo* or *in vitro* prompted the experiments with rat diaphragm pieces. The diaphragm of young (200 grams) rats is sufficiently thin to allow adequate diffusion of oxygen (11) and constant oxygen consumption is maintained for well over two hours. One disadvantage of using the rat diaphragm is that its small size prevents the use of more than duplicate determinations from a single rat. The difference of 6.6 per cent between the  $Q_{O_2}$  values obtained from diaphragm pieces of curarized and control rats, and the  $t$  value of 1.427 indicate that, as in the dog, d-tubocurarine has no significant effect on the oxidative reactions in fairly intact preparations of rat muscle.

Since the diaphragm is usually the last muscle to be paralyzed by curare (9), strips of rat sternohyoid muscle were employed to check further the possibility of a species difference. The data cited earlier show on a limited number of animals that d-tubocurarine has no significant effect on the oxygen consumption of rat sternohyoid muscle strips. The possibility of a species difference seems to have been eliminated.

It remained further to be shown that the mincing of dog muscle would establish the environment necessary to reproduce in this species the increased oxygen consumption due to d-tubocurarine *in vivo* which was noted when rat muscle minces were used. The data provide evidence of such an increase in oxygen consumption, which, as for the rat minces, was accompanied by a greater utilization of pyruvate by the mince of curarized tissue. Again, pyruvate addition was not necessary to a demonstration of the effect. It should be noted that minced dog muscle maintained a fairly constant rate of oxygen utilization until

well into the second hour if substrate was present, and that the difference in rates between curarized and control tissue was much greater during the second hour. The evidence presented on the *in vitro* addition of d-tubocurarine to dog sternohyoid muscle mince, as for the rat, suggests an increase in oxygen uptake, but the 12 per cent increase is of questionable significance, while the 17 per cent increase over the control obtained with mince from the same dog following the injection of d-tubocurarine might be considered significant.

The oxygen consumption of rat brain tissue appeared not to be altered by doses of curare which paralyzed muscles.

The significance of the results of the experiments on muscle reported in this paper is not clear. It appears that a definite increase in oxygen consumption in muscle caused by d-tubocurarine occurs only when the muscle is minced and when the d-tubocurarine has been injected into the animal before the muscle is removed. There is a possibility that the d-tubocurarine molecule is changed in some way before it acts in the body, and that this altered molecule is the actual compound causing the typical curare effects, which, in these experiments, include a marked increase in oxygen consumption. Such an alteration would explain the failure of the alkaloid to cause a response when added to the mince *in vitro*. The fact that the increase is not noted when muscle is studied in a more intact form detracts from the above hypothesis and also detracts from, but does not entirely eliminate, the possibility that d-tubocurarine has an actual physiologic action on the oxidative reactions of mammalian muscle.

#### SUMMARY

Minces of rat and guinea-pig gastrocnemius and dog sternohyoid muscles consumed significantly greater amounts of oxygen than controls following the *in vivo* administration of d-tubocurarine, but not following the *in vitro* addition of the compound to the reaction vessels. The greater oxygen consumption was noted regardless of the presence of added pyruvate as substrate, but when present, greater amounts of pyruvate were utilized. Oxygen consumption of strips of dog and rat sternohyoid muscles and pieces of rat diaphragm, which approach more closely the natural physiologic state, was not altered significantly by d-tubocurarine *in vivo* or *in vitro*. Cyclopropane anesthesia, calcium ions, and anoxia were eliminated as factors in the responses obtained. It seems probable that d-tubocurarine, in doses sufficient to paralyze respiration, has no toxic effect on the rate of oxidative reactions in intact muscles or brain.

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# ON THE ELECTROTONIC NATURE OF STIMULATION, INHIBITION, SUMMATION AND AFTER-DISCHARGE OF NERVE CENTERS<sup>1, 2</sup>

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The electrotonic theory of nervous integration (Gesell, 1940) depends on two facts—the existence of neuro-cellular electrotonic currents and the rhythmical response of living cells to such currents. The theory holds that neuro-cellular potential gradients cause current to flow within the nerve cell from the dendrites to the axon hillock. Absence of potential gradient and high resistance in the neuroaxon proper forces an outflow of the neuro cellular current through the axon hillock. At this point the neuromembrane is rhythmically activated at a frequency determined by the intensity of the current.

Neuro-cellular potential gradients conceivably originate in several ways; from neuro-cellular metabolic gradients, from membrane permeability gradients, from a fused summation of action potentials impinging on the neuron and from acetylcholine liberated by these impulses. The experiments of Loewi, and Dale and their associates and many other workers have given special emphasis to the rôle of acetylcholine in nervous integration.

High content of cholinesterase in the electrical organ of the fish and the capacity of that organ to generate high voltage currents (Nachmansohn, Cox, Coates and Machado, 1942) provide indirect but forceful evidence for the electrogenic function of acetylcholine. Excitation of the cerebral cortex, the respiratory center, isolated ganglion cells and striated muscle by exogenous acetylcholine indicate the possibility of a broad utilization of acetylcholine by the body. Rhythmical responses to electrotonic currents of inorganic nerve models, of plant cells, and of nerve and muscle fibers (Lillie, Osterhout and Hill, Adrian, Gasser and others) indicate the probability of a functional utilization of electrotonic currents physiologically generated by acetylcholine. Graded response of the respiratory center to increasing injections of acetylcholine via the vertebral artery suggest that the amount of free acetylcholine liberated physiologically in nerve cells may control the intensities of electrotonic currents and thereby the degree of activity of nerve centers. Greater summation and after-discharge of spinal reflexes resulting from prolongation of sensory stimulation such as described by Sherrington, is conceivably the result of a greater accumulation of acetylcholine.

**PROCEDURE.** With these ideas in mind we attempted to study the laws of stimulation of nerve centers by recording respiratory reflexes of the vagus, superior laryngeal, saphenous, and carotid nerves. These nerves were stimulated

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<sup>2</sup> Supported in part by the John and Mary Markel Foundation.

faradically with a duBois Reymond induction coil in which the hammer vibrated at a frequency of about 45 per second. Stimulations were varied in intensity and duration, some were interrupted at regular intervals for periods of approximately one second. Depth and frequency of breathing were registered in the usual way with the aid of a Hutchinson spirometer and rebreathing tank, upstroke indicating inspiration. Dogs, anesthetized with morphine and urethane, were used exclusively.

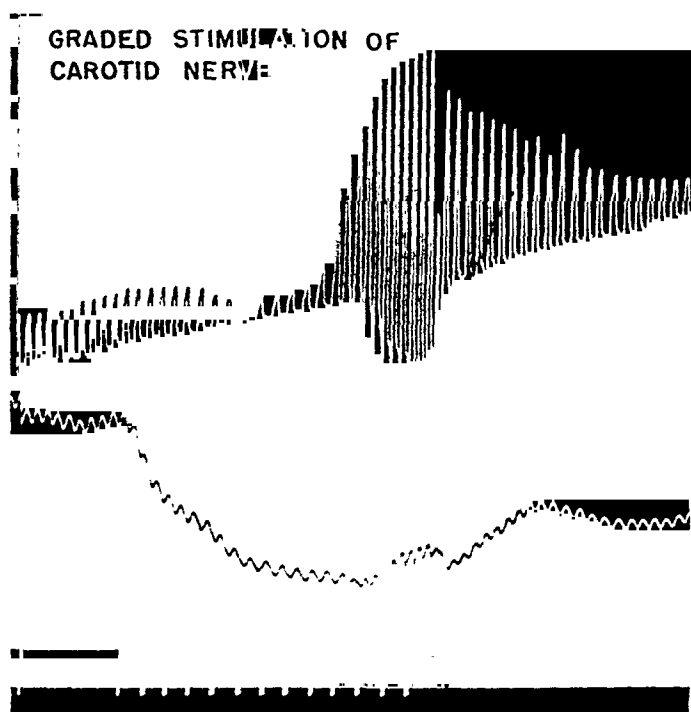


Fig. 1. Respiratory response to graded faradic stimulation of the carotid nerve. Records from above downward are spirometer tracing of respiration, mean blood pressure, duration of stimulation, and times at which the coils of the inductorium were approximated. Inhibitory effects of weak stimulation are limited almost exclusively to inspiration, excitatory effects of medium stimulation appear limited to inspiration. Strong stimulation augments both inspiration and expiration but primarily inspiration, and leaves an after-discharge mainly of the inspiratory half-center.

**RESULTS.** *Physiological characteristics of the carotid nerve.* The carotid nerve proved to be an exceptionally useful nerve for studying the laws of stimulation of the respiratory center. Its essential characteristics are, therefore, illustrated in figure 1. As is well known, this nerve contains two sets of afferent fibers—*respiratory excitatory*, arising in the chemoceptors of the carotid body; and *respiratory inhibitory*, arising in the stretch receptors of the carotid sinus (Heymans). We have found these two sets of fibers to be susceptible to a certain degree of selective stimulation by gradation of intensity of shocks. Weak faradic stimulation, for example, which begins at the left of figure 1 diminishes the depth of breathing. With medium and strong stimulation the excitatory effects of the chemoceptor afferents overpower the inhibitory effects of the sinus

afferents. Medium stimulation increases the depth of inspiration. Strong stimulation increases the force of both inspiration and expiration.

Since the usual effect of strong stimulation is predominantly inspiratory, each chemoceptor afferent may be shown as dividing into two branches—one branch terminating with more synapses at the inspiratory neurons than the one terminating at the expiratory neurons (see fig. 11). Such dual excitation is thought to be a common characteristic of the respiratory afferents of the vagus, superior laryngeal, saphenous and other nerves. The excitatory effects exerted on breathing by individual afferents would then depend upon the pattern of differential termination at the inspiratory and expiratory half-centers.

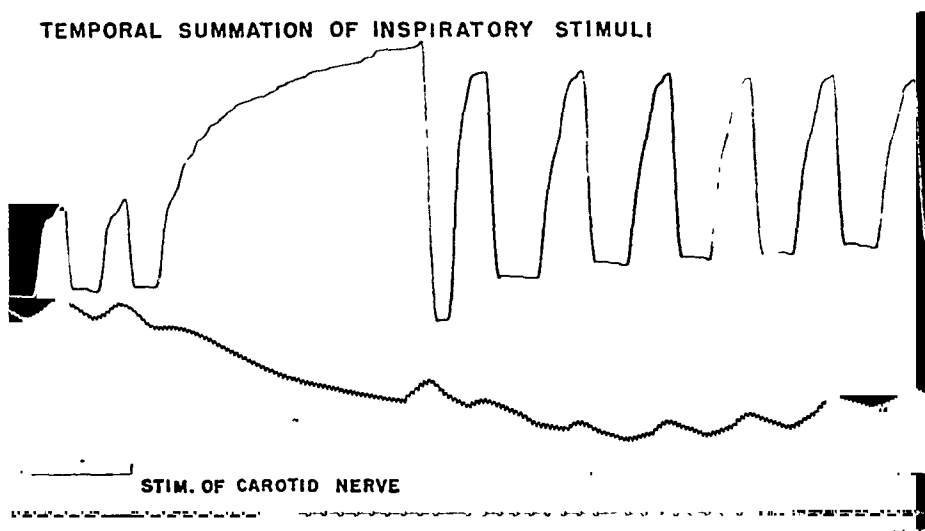


Fig. 2. Respiratory response to faradic stimulation of carotid nerve, after double vagotomy. Duration of stimulation 20 seconds. Note progressive increase of strength of inspiration indicating a prolonged temporal summation of stimuli in the inspiratory half-center. Note persistence of after-discharge of the inspiratory half-center.

*Temporal summation of stimuli in the inspiratory half-center.* The effects of stimulation of the carotid nerve are greatly modified by vagal reflexes. With the vagus nerves intact, stimulation of the carotid nerve shortens the duration of both inspiration and expiration and thus increases the frequency of breathing. When the vagal reflexes are eliminated by double vagotomy, stimulation of the carotid nerve produces a lesser quickening of breathing as in figure 6, a slight slowing of rhythm as in figure 7, or occasional marked prolongation of inspiration as in figures 2 and 4. Such prolonged inspirations offer unusual opportunity for studying temporal summation of stimuli at the inspiratory half-center. As is indicated by the increasing depth of inspiration in figure 2, the activity of the inspiratory half-center increases for a period of eighteen seconds. This increasing activity of the inspiratory half-center automatically produces a progressively increasing reciprocal inhibition of the expiratory half-center by the mechanism represented in figure 11, thereby delaying active expiration and a consequent interruption of the inspiratory act. The progressive increase in depth of inspiration is presumably an expression of summation of stimuli at the

inspiratory half-center which, in terms of the humoro-electrotonic theory, may be interpreted as a gradual accumulation of acetylcholine at the excitatory poles of the inspiratory neurons. The curve of inspiration may then be tentatively regarded as an index of the amount of acetylcholine accumulated in the inspiratory half-center.

*Temporal summation of stimuli in the expiratory half-center.* Just as stimulation of the predominantly inspiratory carotid nerve throws the respiratory center off balance in favor of the inspiratory half-center, so stimulation of a predominantly expiratory nerve, such as the superior laryngeal, throws the center

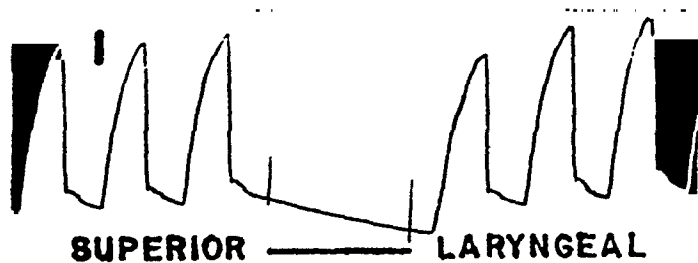


Fig. 3. Respiratory response to faradic stimulation of superior laryngeal nerve. Note progressive increase of strength of expiration indicating a progressive temporal summation of stimuli in the expiratory half-center (fig. 1, Gesell and Hamilton, 1941).

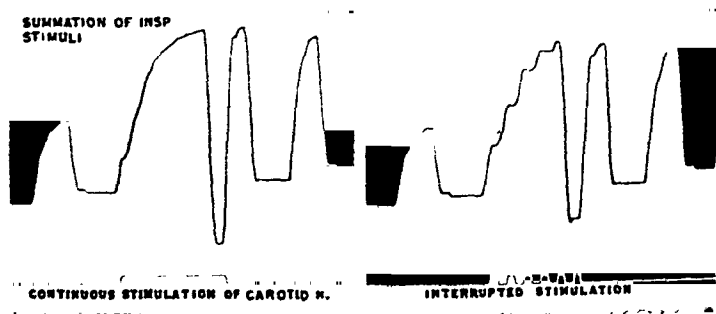


Fig. 4. Comparisons of inspiratory response to continuous and to interrupted faradic stimulation of the carotid nerve. With interrupted stimulation, note the step-like increase of inspiration suggesting a step-like accumulation of acetylcholine in the inspiratory neurons.

off balance in favor of the expiratory half-center (see figs. 3 and 11). Since increased activity of the expiratory half-center produces correspondingly powerful reciprocal inhibition of the inspiratory half-center, temporal summation of stimuli is allowed to continue at the expiratory half-center for an abnormally long period of time. The progressive increase in depth of expiration is presumably due to this summation. The curve of expiration may then be tentatively regarded as an index of the amount of acetylcholine accumulated in the expiratory neurons.

*Periodically interrupted temporal summation of stimuli.* Periodic interruption of faradic stimulation of the carotid nerve modifies the character of the inspiratory response in a most interesting way. In the first record of figure 4, for exam-

ple, where the nerve is stimulated continuously for a period of eight seconds, the curve of inspiration rises smoothly in its usual manner. In the second record, however, where stimulation is periodically interrupted for periods of approximately 0.75 second duration, the inspiratory contraction rises in a step-like manner. This suggests a step-like accumulation of neuro-humor in the inspiratory half-center.

The course of the curve during the periods of interruption of stimulation calls attention to the possibility that acetylcholine is very slowly destroyed in the respiratory center, a view which is compatible with the electrotonic theory of nervous integration but incompatible with the theory of *transmission* of nerve impulses. It will be recalled that slowness of destruction of acetylcholine was raised as an objection to humoral mediation.

Periodic interruption of faradic stimulation of the superior laryngeal nerve produces a comparable step-like increase of expiratory contractions suggesting a step-like accumulation of acetylcholine in the expiratory neurons. (See fig. 5.)

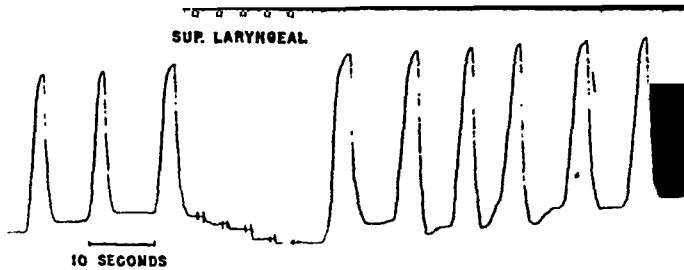


Fig. 5. Step-like increase of strength of expiration produced by a periodically interrupted faradic stimulation of the superior laryngeal nerve. (Fig. 78, Gesell, 1940.)

*Simultaneousness of temporal summation of stimuli in the inspiratory and expiratory half-centers.* The striking correspondence of the course of temporal summation of stimuli in the inspiratory and expiratory half-centers seen in figures 2, 3, 4 and 5, implies a similarity of the mechanism of stimulation in both half-centers. Associated increase of inspiration and expiration so commonly seen in reflexogenic hyperpneas (figs. 1, 6 and 7) indicates a simultaneous temporal summation in both inspiratory and expiratory half-centers. Figure 6 inquires into the rôle of simultaneousness of summation of stimuli. Graph 1 represents the selective summation of stimuli at the inspiratory half-center which was observed in figure 2 on stimulating the carotid nerve. Graph 2 represents the selective summation of stimuli at the expiratory half-center which was observed on stimulating the superior laryngeal nerve in figure 3. Graph 3 represents the *theoretically* expected simultaneous summation of inspiratory and expiratory stimulations. These graphs may now be compared with the actual results (graph 4) obtained by stimulating a dual excitatory nerve such as the carotid nerve. Connecting the crests of inspiration, as we have done, reveals an inspiratory curve of summation similar to graph 1. Connecting the troughs of expirations reveals an expiratory curve of summation similar to graph 2. Taken

together these curves present a picture comparable to graph 3. In terms of the humoro-electrotonic theory, the inspiratory and expiratory curves of summation of record 4 indicate a simultaneous accumulation of acetylcholine in the opposing half-centers in conformity with our schema illustrated in figure 11. That is tantamount to implying an omnipresence of electrochemical forces at both half-centers. But the existence of electrochemical forces in both half-centers must tend toward simultaneous contractions of inspiratory and expiratory muscles. How, then, is co-ordinated utilization of simultaneously existing inspiratory and expiratory electrotonic currents brought about? Alternating reciprocal inhibition such as originally proposed by Brown (1911 and 1912) is held to be the mechanism involved. The manner in which this mechanism is thought to work is illustrated in figure 11 and described in our discussion..

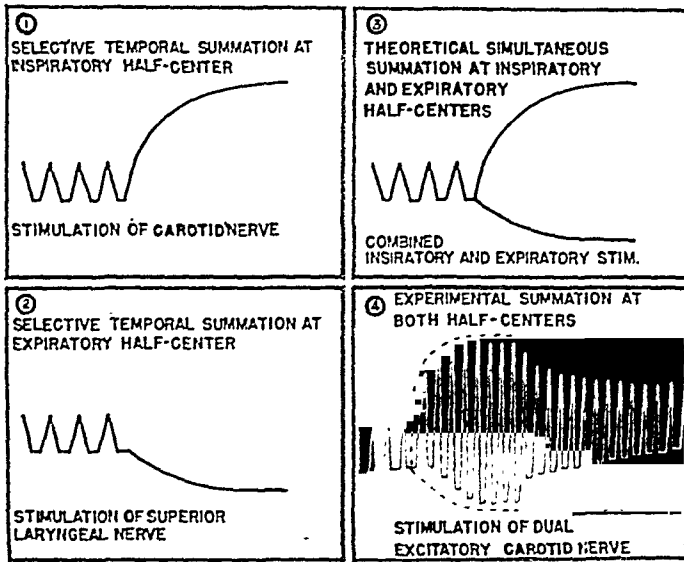


Fig. 6. Analysis of the electrotonic concept of simultaneousness of temporal summation of stimuli at the inspiratory and expiratory half-centers. The gradual accumulation of acetylcholine in the inspiratory and expiratory half-centers as indicated by the broken white curves of graph 4 generates the electrotonic energy producing co-ordinated alternating activity of the half-centers. (See text for further explanation.)

*Spatial summation of stimuli.* Faradic stimulation of both left and right carotid nerves produces greater hyperpnea than does stimulation of either nerve alone (see fig. 7). This is an example of the well known phenomenon of spatial summation of stimuli and should be differentiated from temporal summation illustrated in figures 2 and 3. It is pertinent to note that spatial summation occurs simultaneously at the inspiratory and expiratory half-centers, since expirations as well as inspirations are increased in force. In terms of humoral mediation this spatial summation means that more acetylcholine is liberated in the respiratory center with bilateral stimulation than by stimulation of one nerve alone. It may be assumed that left and right chemoceptor afferents terminate in the manner indicated in figure 11.

*Comparison of the nature of inhibition and stimulation.* We have shown that

weak stimulation of the carotid nerve produces inhibition of breathing. Such selective stimulation of the inhibitory afferents originating in the carotid sinus fortunately affords an opportunity for comparing the nature of inhibition with that of stimulation. In figure 8 it will be noted that faradic stimulation of the carotid nerve produces a *gradual diminution* of breathing comparable in course to the *gradual increase* of breathing produced by stimulation of the excitatory fibers of the carotid nerve in figure 7. Thus, inhibition and stimulation are shown to be similar with respect to temporal summation of stimuli.

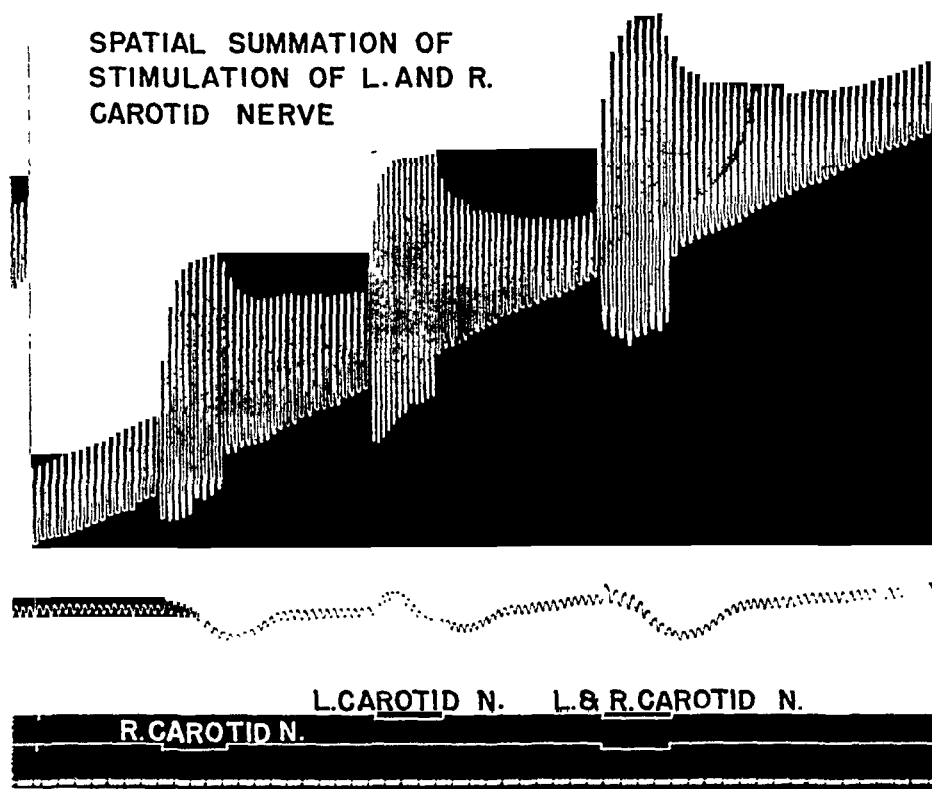


Fig. 7. Spatial summation of stimuli produced by combined faradic stimulation of left and right carotid nerves. Spatial summation occurs simultaneously in the inspiratory and expiratory half-centers since the depths of both inspiration and of expiration are increased.

Combined stimulation of the inhibitory fibers of the left and right carotid nerves (see fig. 8) produces greater inhibition of breathing than does stimulation confined to one nerve alone, which reveals a similarity of inhibition and stimulation with respect to spatial summation of stimuli.

Inhibition of breathing produced by weak stimulation of the carotid nerve is followed by a gradual increase in depth of breathing which is comparable to the gradual decrease in depth of breathing following excitatory stimulation of the carotid nerve (compare figs. 8 and 7). The gradualness of recovery of both inhibitory and excitatory reflexes reveals a similarity of after-discharge of inhibition and stimulation.

It is interesting that the inhibition of eupnea noted in figure 8 is confined almost exclusively to the inspiratory contractions. Inhibition of expiration is



barely visible. The insusceptibility of eupneic expiration to inhibition seems related to the lack of activity of the expiratory muscles, for when the activity of these muscles is increased, as it is in hyperpnea, expiration becomes highly susceptible to inhibition. Witness the symmetrical inhibition of inspiration and expiration in figure 9 where the inhibitory afferents of the carotid nerve are stimulated by inflation of the sinus.

*Comparison of the nature of response of respiratory and circulatory centers.* Comparison of respiratory and circulatory reflexes elicited through the carotid nerves suggests that the respiratory and circulatory centers function in similar ways (see figs. 7 and 10). The well known *gradual* fall of blood pressure in figure 10, (see also Bayliss) is similar to the *gradual* increase of breathing in

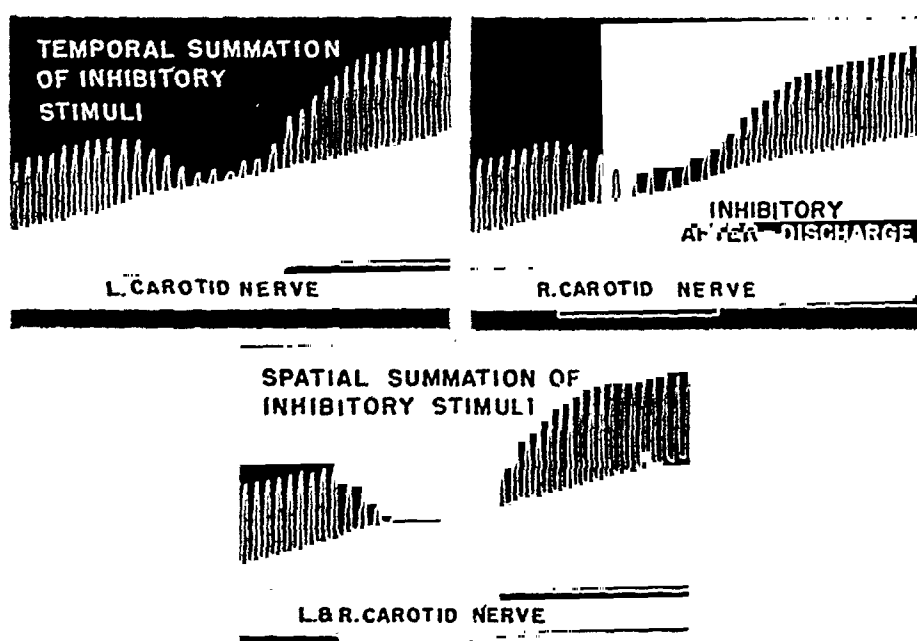


Fig. 8. Temporal and spatial summation of inhibitory impulses and inhibitory after discharge, produced by weak faradic stimulation of the carotid nerve.

figure 7, both indicative of a *gradual* summation of stimuli in the respective centers. The gradual rise of pressure after stimulation has ended is also similar to the gradual decrease of respiration after excitatory stimulation of the carotid nerve has ended, both indicative of a similarity of after-discharge in the respective centers. The greater drop of pressure produced by combined stimulation of the left and right carotid nerves as compared with that of single nerve stimulation reveals a spatial summation of stimuli in the vasomotor reflex similar to that observed in the respiratory reflexes. Granting humoro-electrotonic integration for these widely differing reflexes, the pertinent point illustrated by these comparisons is that electrotonic integration is as suitable for the control of rapid respiratory movements as for the more sluggish changes in blood pressure where smooth musculature is involved.

DISCUSSION. *Schematic summarization of the electrotonic theory of nervous integration.* Our theory of the electrotonic nature of nervous integration is tentatively summarized in our working schema of the respiratory center (see fig. 11). The inspiratory and expiratory half-centers are represented by single neurons, an inspiratory neuron on the left and an expiratory neuron on the right. For purposes of simplicity internuncial cells are omitted. Stimulatory impulses are envisaged as impinging directly on the inspiratory and expiratory neurons by a great variety of respiratory afferents. Only one chemoceptor afferent and its arrangement of synapses, however, is shown. In agreement with

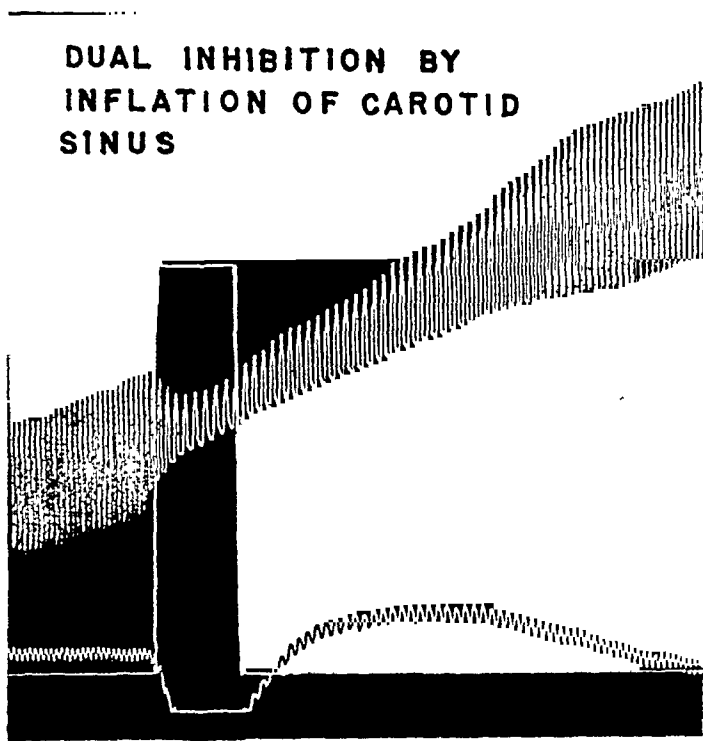


Fig. 9. Inspiratory and expiratory inhibitory effects of inflation of the carotid sinus in a hyperpneic animal *when expirations are active*. Compare with the dominantly inspiratory inhibitory effects of the carotid nerve during eupnea in figure 8 when expirations are mainly passive.

the dominant inspiratory effect of the chemoceptors four synapses are arbitrarily allocated to the inspiratory neuron and two synapses to the expiratory neuron. Dominantly expiratory afferents coming from the vagal stretch receptors would in contrast have predominant expiratory allocation of synapses.

Highly electrogenic acetylcholine liberated by impulses impinging on the dendrites and cell bodies establish neurocellular electrotonic currents represented by the broken line circuits running through each neuron and its environment. Intracellularly these currents concentrate as they approach their exit at the axon hillock. At this point of concentration the outflowing current sets up

rhythmical discharges of the membrane of the axon hillock and thus generates the respiratory motor impulses which control the respiratory act.

Acetylcholine liberated by impulses impinging at the axon hillock creates a localized negativity which opposes the excitatory electrotonic current originating in the dendrites. The excitatory current passing through the membrane of the axon hillock is thereby weakened, and the normal generation of nerve impulses is inhibited. In agreement with this concept, inhibitory afferents such as the carotid sinus afferents and reciprocating inhibitory collaterals terminate at the axon hillock (see fig. 11). Central neurons are accordingly divided into two functional zones designated as excitatory and inhibitory poles.

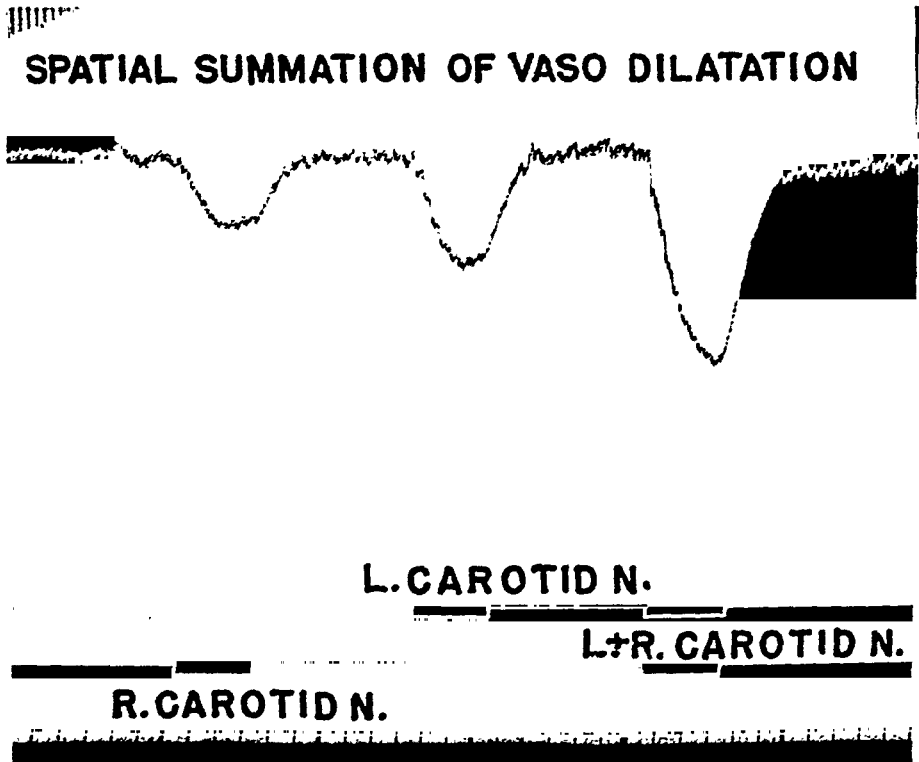


Fig. 10. Temporal and spatial summation of inhibitory stimuli in vasomotor center. See text.

Myriads of impulses impinging and summing continuously at the excitatory poles of the inspiratory and expiratory neurons tend to stimulate both half-centers simultaneously. Such uncoordinated activity, however, is prevented by the interconnecting reciprocal inhibitory collaterals, since activity of one half-center automatically inhibits the activity of the other sending impulses to the inhibitory poles of its antagonistic neurons. It should be kept in mind, however, that reciprocal inhibition has an additional accompanying effect of paramount importance. If we refer to the two half-centers in figure 11 as (A) and (B) respectively, reciprocal inhibition of the half-center (A) prevents impulses impinging on that center from reaching threshold stimulating value, thus holding half-center (A) temporarily in abeyance. Abeyance of half-center (A), in turn,

allows active half-center (B) to remain open to immediate stimulation by all impulses continuing to impinge on half-center (B) so long as half-center (B) is active. In other words, precedence of *immediate stimulation* is automatically conferred upon that half-center which is active. Reciprocal inhibition and reciprocal precedence of stimulation working hand in hand accomplish the coordinated alternating activity of normal breathing.

What then is the evidence for the existence of neuroelectrotonic currents and for an anatomical arrangement consistent with our theory of nervous integration?

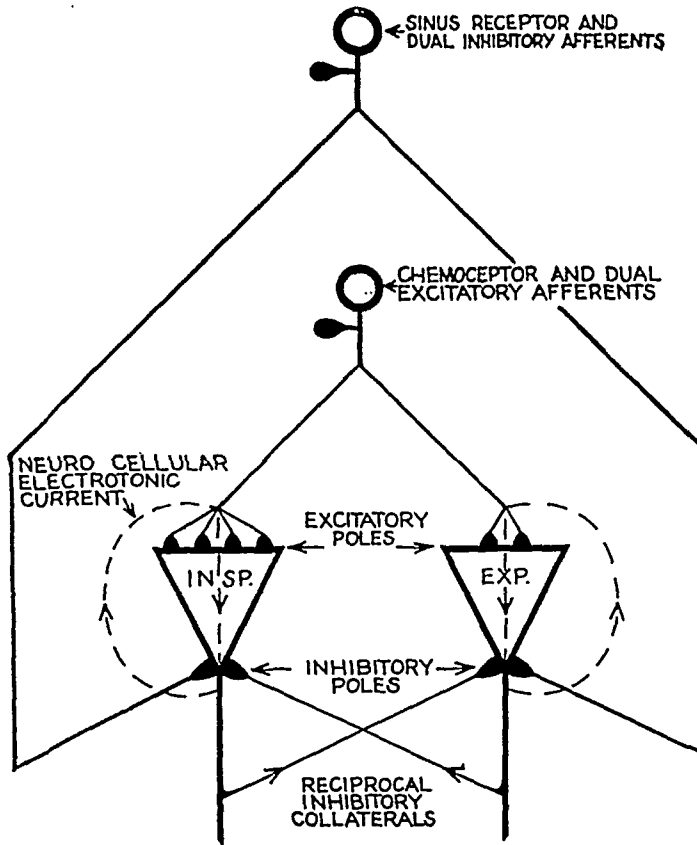


Fig. 11. Simplified schema of the respiratory center illustrating our concept of electrotonic integration of a paired motor act. Structural basis of dual stimulation, dual inhibition, reciprocal inhibition and precedence of stimulation. (For details see discussion.)

*Direct evidence on the existence and on the effects of neuroelectrotonic currents.* While it is true that our present evidence for humoral mediation is of an indirect nature, the concept of electrotonic mediation is importantly supported by direct, crucial observations. The existence of neurocellular electrotonic currents has been definitely established by Barron and Matthews (1938) and by Eccles (1943). Furthermore, the activity of ventral horn nerve cells has been shown by Barron and Matthews (1938) to be proportional to the intensity of the slow negative potentials of the ventral root. In their figure 9A they show the effects of a gradually increasing pressure on the foot followed by a

sudden release of that pressure. As the pressure increases the slow negative potential and the impulse discharge of the ventral root increases. According to our interpretation increasing pressure on the foot of the frog should lead to an increasing bombardment of the ventral horn cells, a corresponding accumulation of acetylcholine at these cells, increasing electrotonic currents, and an increasing discharge of the motor nerve cells. When pressure on the foot is released the slow negative potential and impulse discharge of the ventral root diminish.

*Anatomical basis of electrotonic theory of stimulation and inhibition.* Neurohistologists were the first to be impressed with the possible significance of the axon cap of Mauthner's cell. This structure suggested a division of the function in the nerve cell, in which stimulation would originate in the dendritic pole and inhibition in the opposite pole of the neuron. This concept is all the more interesting in the light of the opinion of Bartelemesz (1915), Beccari (1907) and Tello (1909) that Mauthner's cell is a nucleus in itself for it possesses more connections than the associated groups of the nucleus motorius tegmenti. Two nerve cells, the left and right Mauthner's cells, working together might then constitute the simplest conceivable nerve center comparable to that of our figure 11 (Gesell, 1940).

Bodian (1938) comments on the axon cap of Mauthner's cell as follows: "It is conceivable that there is here at the origin of the axon an inhibitory mechanism similar to that suggested by Coghill (1934) at the terminals of the collaterals of Mauthner's fibers on the motor cells of the spinal cord. These collaterals are also in synaptic relation with the *axon necks* of secondary cells rather than with their dendrites or perikarya (Beccari, 1907). It is interesting to note that spiral axo-axonic terminals have been described by Pitzorno (1913-14) in the ciliary ganglion of selachians and chelonians." With these suggestions in mind Gesell (1940) proposed that the basket enclosing the body of the Purkinje cell might exemplify still another inhibitory arrangement similar to that proposed for the axon cap of Mauthner's cell. Far more significant, however, is the more recent observation of Bodian (1942) that extension of synapses onto the axon hillock represents the most common pattern of synaptic distribution in the vertebrates. A simpler structural arrangement for the control of excitation and inhibition of nerve cell activity than a gross topographical concentration of excitatory and inhibitory synapses is difficult to imagine.

*Sources of neuro electrotonic currents other than acetylcholine.* While the electrogenic properties of acetylcholine and its relatively slow destruction seem to meet the requirements of the electrotonic theory of nervous integration, it was other considerations which originally suggested the electrotonic theory. The myriads of impulses bombarding individual nerve cells, the great variety of fibers terminating on many cells, and the disorderly and asynchronous nature of bombardment when taken together seemed to contradict the theories of impulse conduction by nerve cell bodies and to favor the *generation* of impulses by the neurons instead. In view of the experiments of Barron and Matthews, of Eccles and of Fessard (1936), the electrotonic theory is well sup-

ported by direct evidence. More direct evidence for the source or sources of electrotonic currents, however, is highly desirable.

The possibility of sources other than acetylcholine should be considered. Increased activity of the neuron, for example, might increase existing metabolic and permeability gradients and produce the effects of summation of stimuli described above and retarded recovery of the nerve membrane after hyperpnea to its normal permeability and metabolic gradient could result in the after-discharges illustrated above. The fact that the after-discharge of reflex hyperpnea varies inversely instead of directly with the extent of hyperpnea, on the other hand, leaves little support for this hypothesis. Agreement of this inverse relationship with the acid-humoral theory of respiratory control (Gesell, Brassfield and Hansen, 1942; Gesell and Hansen, 1945) would seem to strengthen the concept that acetylcholine may be the main source of electrotonic currents.

#### SUMMARY AND CONCLUSIONS

Strong faradic stimulation of the predominantly inspiratory carotid nerve, in the vagotomized dog, occasionally produced initial inspirations of great duration and of progressively increasing strength. Such inspirations were regarded as indication of sustained temporal summation of stimuli in the inspiratory half-center.

Stimulation of a predominantly expiratory nerve such as the superior laryngeal produced expiratory contractions of similar characteristics indicating sustained temporal summation of stimuli in the expiratory half-center.

The degree of temporal summation of stimuli was related to the intensity of neuro-cellular electrotonic currents which were held to vary with the amount of acetylcholine accumulated at the excitatory poles of the inspiratory and expiratory neurons respectively.

When stimulation of either the carotid or superior laryngeal nerve was interrupted at regular intervals of approximately one second duration, inspiratory and expiratory contractions increased in step-like manner instead of smoothly, suggesting an interrupted accumulation of acetylcholine in the half-centers of respiration.

Sustained stimulation of the carotid nerve producing rhythmic breathing showed a progressive increase of strength of both inspiration and expiration as stimulation advanced. Such simultaneous augmentation of both inspiration and expiration suggested a simultaneous accumulation of acetylcholine in both half-centers. Simultaneous contractions of inspiratory and expiratory muscles were believed to be prevented by the intervention of reciprocating collaterals which provide for co-ordinated alternating activity of half-centers under steady electrotonic drive.

Spatial summation of stimuli, demonstrated for the inspiratory and expiratory half-centers by combined stimulation of the left and right carotid nerves, was explained, like temporal summation, in terms of increased liberation of acetylcholine in the respiratory half-centers.

Simultaneous inhibition of both inspiration and expiration produced by weak

stimulation of the carotid nerve agreed with a dual inhibitory function of the sinus afferents.

The similarities of temporal and spatial summation of stimuli and of reflex after-discharge of inhibition and stimulation suggested the possibility that acetylcholine is responsible for inhibition as well as stimulation of nerve centers.

This dual function of a single neuro-humor was held to depend upon a topographical termination of the excitatory and inhibitory afferents in the general region of the excitatory and inhibitory neuron poles respectively. Anatomical evidence is cited for this concept.

Basic similarities of respiratory and blood pressure reflexes implied a common electrotonic mechanism of integration of two widely differing physiological systems and suggested that electrotonic integration is adaptable to the control of both brisk and sluggish e d-organs.

*Addendum.* Since this paper has gone to press, a misunderstanding seems to have arisen concerning the Electrotonic Theory of Nervous Integration proposed in "A Neurophysiological Interpretation of the Respiratory Act," Gesell, 1940. See footnotes on pages 619 and 650 of Pitts' Physiological Reviews paper, 1946, which are here quoted: Footnote 1: "This concept of polarization of the nerve cell body was first clearly expressed by Gerard, 1932 and 1942." Footnote 2: "The basic concept of excitatory poles of the neuron had been *developed* previously by Gerard 1932 and 1942 (*italics ours*) and is equally applicable whether the synaptic excitor is humoral or electrical."

The statement of Gerard (Physiol. Rev., 1932), to which Pitts is presumably referring, follows: "Since impulses reaching spatially separated dendrites of a single motoneuron do summate in producing motor discharges, there must be some common locus of action; and some agent must carry the separate changes to this region. If a potential change sweeping along surfaces and leaving a relatively lasting diminution in polarization is imagined, a picture not unlike conduction in the nerve fibre emerges. But a similar, if inverse, change must be assumed to follow the arrival of an inhibitory impulse. This appears to require a second, qualitatively different, membrane change propagating along the same membrane as the excitatory one and leading to opposite polarization effects. Though *not inconceivable*, such a system certainly has no known prototype and is not very attractive. (A more encouraging situation *would* result *if* there existed a spacial separation of excitatory and inhibitory endings on opposite poles of a motoneurone (*italics ours*). The same local change in each group might lead in one case to increase, in the other to decrease, of a pre-existing polarization or other gradient along the cell, and so its state of activity. Adrian's study of the isolated nervous system of the water beetle (13) shows spontaneous, slow and rhythmic changes in the potential difference between ganglion cells and their axones. This, however, he considers as a consequence of changes in the central excitatory state rather than as the state itself). A chemical mechanism and diffusion would not lead to such difficulty, and it is tempting to consider here specific possibilities."

This parenthetical remark, qualified as it is, seems to suggest that polar function had been excluded by Gerard in favor of more promising theories of nervous integration. Be that as it may, the idea of polar function, to the best of our knowledge, did not reappear in neurophysiological literature until publication of The Electrotonic Theory of Nervous Integration by Gesell, 1940.

Neuro-anatomists had, however, on a purely structural basis, suggested, as cited in our earlier papers, a bipolar function of neurones with excitation occurring at one end and inhibition at the opposite end. It is the directness of this suggestion, the reasonableness of Brown's concept of half-centers, coupled with the probable existence of a simple electrotonic mechanism of excitation and inhibition, plus the apparent untenability of the electrical theory of *synaptic transmission of impulses* that call for a new outlook in the field of nervous integration.

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## APPLICATION OF THE RADIOACTIVE RED CELL METHOD FOR DETERMINATION OF BLOOD VOLUME IN HUMANS<sup>1, 2, 3, 4</sup>

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Many methods have been devised for the determination of the volume of the blood in humans. Objections have been raised to each of these, some based upon observation, some largely theoretical. One of the most controversial issues has been the discrepancy between the volume determined by plasma dye methods and the volume determined by direct red cell methods, in each case the opposite fraction being determined by difference through the hematocrit ratio. Whipple and his collaborators first noted this discrepancy many years ago (1).

Hahn and his collaborators described a method of determining the red cell mass in dogs using radioactive red cells from a donor dog (2, 3). For all practical purposes this method is identical with the procedure for humans described below. It is not within the scope of this paper to analyze critically the relative merits of blood volume procedures; however, we would like to point out the applicability of the radioactive iron labeled red cell method to humans and to show that the previously reported discrepancy indeed exists.

**METHOD.** Volunteer normal medical student universal donors who were Rh negative were sent to the blood bank for two 500 ml. phlebotomies a week apart. One week after the second bleeding Iron<sup>59</sup> was administered orally three times a day between meals. About one million countable counts per minute

<sup>1</sup> The work described in this paper was done under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Vanderbilt University.

<sup>2</sup> Thayer General Hospital patients were studied through the co-operation of Brigadier General Henry C. Pillsbury, Officer Commanding, Lt. Col. Richard Stetson, Chief of the Medical Service, Major Donald Ferguson, Chief of the Reconditioning Service and other members of the staff.

<sup>3</sup> Equipment used in this research was available through grants by the Nutrition Foundation and Ciba Pharmaceutical Products, Inc.

<sup>4</sup> The clinical portion of this work was done in the Lung Station of the Department of Medicine, a laboratory organized under a grant from the Commonwealth Foundation.

<sup>5</sup> Present address: Camp Detrick, Frederick, Maryland.

were administered in each dose. A total of 25 million counts were thus administered. The blood activity rose to about 1000 counts per minute per ml. of donor blood. Booster doses of iron were administered occasionally to maintain this level of activity. Bleeding of donors was restricted to 100 ml. per week.

Patients were in the basal state. For plasma volume determination we followed essentially the technique of Gibson and Evans (4). Evans blue dye was administered with a 10 ml. calibrated syringe immediately followed by freshly drawn citrated radioactive donor blood with a 50 ml. calibrated syringe. A moiety of the blue dye and of the blood-citrate mixture were reserved. To have a readily visible reference point a line was engraved on the syringe piston and brought opposite the barrel calibration marks. Volumes of water contained by these syringes could be reproduced consistently within 0.2 per cent. After fifteen minutes a series of serum samples were drawn and delivered into oiled tubes at accurately timed intervals of about 5 minutes for blue dye determination. Usually six whole blood samples were delivered into 15 ml. calibrated centrifuge tubes containing 2 ml. of 3 per cent sodium citrate. These were centrifuged and the hematocrit ratio was calculated. The plasma was then discarded. Tubes were paired at random and each pair was pooled to supply an adequate amount of cells for radioactivity determinations.

Dye determinations were made on serum samples read in a photoelectric colorimeter with a 620 filter against a distilled water blank. (Readings at 540 were also made to evaluate hemolysis but these readings were not used in calculation.) Four 1:400 distilled water dilutions of the same ampule of dye were also read, as was the blank serum sample. The calculation was:

$$\frac{Rs \times 400 \times Vd}{Rde - Rbs} = \text{Plasma Volume}$$

Where: Rs = Average reading of the 4 standards.

Vd = Volume of dye contained in calibrated syringe.

Rde = Extrapolation point of dyed serum sample readings.

Rbs = Reading of blank serum.

The colorimeter gave linear readings for dye dilutions over the range used. We have found that the reading of dye diluted in serum minus blank serum reading equals the reading of the same concentration of dye in distilled water. The pooled red cell samples were wet ashed, the iron precipitated, redissolved, electroplated and counted on the Geiger-Mueller apparatus (5), as were appropriate aliquots of the donor-blood-citrate mixture. All samples were adjusted to equivalent iron content by appropriate addition of non-radioactive iron prior to ashing to avoid variable self absorption. The calculation was:

$$\frac{Adm \times F \times Vb}{A_{rem}} = \text{Red Cell Mass}$$

Where: Adm = Activity of aliquot of donor blood-citrate mixture.

F = Aliquot factor.

$V_b$  = Volume of donor blood-citrate mixture injected.

$A_{rem}$  = Activity of recipient cells per ml.

For purposes of comparison the data was calculated as though only the blue dye data were available and also as though only the radio red cell data were available. Thus "dye cell volume" was calculated from dye plasma volume and venous hematocrit and "radio plasma volume" was calculated from radio cell volume and the venous hematocrit. "Dye" and "radio total blood volumes" were obtained by addition. "True" blood volume was obtained by adding dye plasma volume to radio cell volume and the average body hematocrit was derived from this.

In order to examine the error attributable to technique several studies were carried out in vitro. Five-hundred milliliters of citrated blood were delivered into into an Erlenmeyer flask. To this "recipient" a 1:10 dilution of blue dye was "administered" through a needle with one of the calibrated syringes currently

TABLE 1

*In vitro experiment to assess the accuracy of the technical phase of the methods used. The experiment is a miniature in vitro reproduction of the procedure employed in patients. Dye concentration and radioactivity were adjusted to simulate levels used in the human subjects*

PLASMA BY VOLUME MEASUREMENT AND HEMATOCRIT RATIO*	PLASMA BY DYE DILUTION	DIFFERENCE	CELLS BY VOLUME MEASUREMENT AND HEMATOCRIT RATIO*	CELLS BY RADIOACTIVE CELL DILUTION	DIFFERENCE
		%			%
383	394	+2.9	174	176	+1.1
372	382	+2.7	169	156	-7.7
362	371	+2.5	164	157	-4.3
352	359	+2.0	159	168	+5.7
341	347	+1.8	155	148	-4.5
331	339	+2.4	150	141	-6.0

\* These volumes are in error by the amount of plasma occluded among the packed cells.

in use in imitation of the technique employed in our patients. The same was done with citrated radioactive donor blood (diluted appropriately with non-radioactive blood) with a calibrated 50 ml. syringe. These dilutions were made to give final dye and radioactivity levels which would correspond with those obtained in the patients. An exactly measured 25 ml. sample was withdrawn and divided between two 15 ml. centrifuge tubes. Ten milliliters of non-radioactive citrated blood was added to the large "recipient" flask. The sampling and addition was repeated five times. A "balance sheet" was drawn up to determine the true volumes involved. The samples were analyzed in the usual way, although of course in this case the colorimetry was done with plasma samples rather than serum. The observations made in one such experiment are presented in table 1.

**MATERIAL.** The 28 male patients were a heterogeneous group. Some were studied when first admitted for therapeutic malaria, others during the course of

TABLE 2

*Twenty-eight determinations of plasma volume and red cell mass in patients in a variety of clinical states*

PLASMA			CELLS			TOTAL				HCT.	
Dye	Radio	Dif.	Dye	Radio	Dif.	Dye	Radio	Dif.	Plasma dye plus radio cells	Average body	Venous
ml.	ml.	liter	ml.	ml.	liter	ml.	ml.	liter	ml.	%	%
2272	2440	-0.17	1452	1560	-0.11	3724	4000	-0.27	3832	40.7	39.0
3316	2845	+0.47	1454	1245	+0.20	4760	4090	+0.67	4561	27.2	30.5
3778	3015	+0.76	1476	1185	+0.31	5254	4200	+1.05	4963	23.8	28.1
3284	2810	+0.47	1252	1070	+0.18	4536	3880	+0.66	4354	24.5	27.6
4480	4640	-0.16	2315	2400	-0.08	6795	7040	+0.24	6880	34.8	34.1
4454	4165	+0.28	2067	1935	+0.13	6521	6100	+0.42	6389	30.2	31.7
3330	2715	+0.61	2815	2295	+0.52	6145	5010	+1.14	5625	40.8	45.8
3027	2230	+0.80	2920	2150	+0.77	5947	4380	+1.57	5177	41.5	49.1
2910	2285	+0.63	2986	2350	+0.64	5902	4635	+1.26	5260	44.6	50.6
3067	2920	+0.15	3086	2940	+0.15	6153	5860	+0.29	6007	48.9	50.1
3167	2290	+0.88	2528	1825	+0.70	5695	4115	+1.58	4992	36.5	44.4
2909	1810	+1.10	2323	1445	+0.86	5232	3255	+1.97	4354	33.1	44.4
2936	2585	+0.35	2422	2130	+0.29	5358	4715	+0.65	5066	42.0	45.2
2928	2130	+0.80	2338	1700	+0.64	5266	3830	+1.44	4628	36.7	44.4
2994	2330	+0.66	2754	2150	+0.60	5748	4480	+1.27	5144	41.7	47.9
3559	3015	+0.54	3483	2105	+1.37	6042	5120	+0.92	5664	37.1	41.1
3430	2155	+0.27	2095	1315	+0.78	5525	3470	+2.06	4745	27.7	37.9
2935	2690	+0.25	1805	1660	+0.15	4740	4350	+0.49	4595	36.1	38.1
3229	2340	+0.89	1693	1260	+0.43	4922	3600	+1.32	4489	28.0	35.0
3070	1915	+1.09	2545	1585	+0.96	5615	3500	+2.11	4655	34.0	45.3
4129	2930	+1.20	2596	1910	+0.69	6725	4840	+1.89	6039	31.6	38.6
3840	2361	+1.48	1442	915	+0.52	5282	3275	+2.01	4755	19.2	27.3
2096	1965	+1.03	1800	1515	+0.28	3896	3480	+0.42	3611	41.9	46.2
2958	2710	+0.25	1497	1400	+0.10	4455	4110	+0.35	4358	32.1	36.5
2727	2757	-0.02	1424	1423	+0.00	4151	4180	-0.03	4150	34.2	34.3
2809	1990	+0.82	1995	1525	+0.47	4802	3515	+1.28	4334	35.1	41.5
3162	2555	+0.60	2064	1795	+0.26	5226	4350	+0.88	4957	36.2	39.5
3226	2860	+0.37	2115	1920	+0.20	5341	4780	+0.56	5146	37.3	39.6
3215	2625	+0.59	2170	1740	+0.43	5348	4350	+1.00	4955	34.9	39.3

*Plasma, dye* is the plasma volume determined by blue dye dilution. *Plasma, radio* is the plasma volume estimated through the venous hematocrit ratio and the red cell mass determined by radioactive red cell dilution. *Cells, dye* is the red cell mass estimated from the venous hematocrit ratio and the plasma volume measured by blue dye dilution. *Cells, radio* is the red cell mass determined by radioactive red cell dilution. *Total, dye* and *Total, radio* are total blood volumes calculated as though the only data available were the venous hematocrit ratio and the dye dilution or the red cell dilution respectively. *Plasma dye plus radio cells* is plasma volume by dye plus red cell mass by radioactive red cells, which presumably represents the best approximation of actual total blood volume. *Hct., average body* is the hematocrit ratio derived from plasma dye plus radio cells. *Hct., venous* is the observed venous hematocrit ratio.

paroxysms, still others during convalescence. It would be impossible from this particular group of patients to arrive at any conclusions with regard to normal individuals. The material is presented, however, because the discrepancies previously observed between the values obtained by circulating red cells and the values obtained by plasma were found. In other words, the venous hematocrit ratio is not observed to be the same as the general body hematocrit (3).

**RESULTS.** The observations made on the patients described in this paper are presented in table 2. Inspection of this table reveals very considerable discrepancies between the results obtained by dye dilution and by radioactive red cell dilution. Particular attention should be paid to the difference between the apparent total blood volume determined by dye and venous hematocrit ratio and that determined by radioactive red cell and venous hematocrit ratio. The majority of the discrepancies are in the same direction, such that volumes as determined by radioactive red cells appear to be considerably smaller than volumes determined by dye. The difference between the average blood volume of 5.35 liters by dye and the average blood volume of 4.35 liters by radioactive red cells is 1 liter, but in individual cases the variation is indeed considerable. It is further seen in all but one instance that the general body hematocrit is lower than the venous hematocrit.

**DISCUSSION.** The method depends for its validity upon a variety of prerequisites. The radioactive red cells administered must mix completely in the circulation (3). They must remain intact because the pigments would be removed from the circulation rather rapidly. When blood is fresh and transfused in good condition, the survival of red cells can be considered to be 100 per cent for the short period of time involved in drawing blood from the donor and administering it to the recipient. There must be no exchange of the radioactive iron in the red cells administered, either with plasma or with other cells (6).

The validity of comparing distilled water diluted dye standards with dye in serum deserves discussion. There are differences in the spectral absorption (7). However, at 620 millimicrons the absorption characteristics differ relatively little (see fig. 7-d of the above cited reference). In practice, with the filter and colorimeter used in this study, only an occasional serum sample shows a significant difference between the reading of dye in water and the reading of the same dilution of dye in serum minus the reading of blank serum. Too much blood would be needed to prepare standard dilutions of dye in serum from each patient. However, the advantages of comparison colorimetry over photometry with most available photoelectric colorimeters are considerable. Finally, since the standards are prepared from the same ampoule of dye actually injected various other possible errors are obviated. Because of these considerations, we prefer to rely upon the comparison procedure rather than upon photometry. The *in vitro* experiment (table 1) was done with dye standards diluted in water just as in the *in vivo* experiments (table 2) and it is evident no substantial error was introduced.

Through the courtesy of Dr. J. G. Gibson, we have had the opportunity to compare our findings with his (8). All of his subjects were normal and it is to

be assumed the circulation was in a much more stable state than in our patients who were in varying stages of disease or convalescence. We believe this accounts for the greater variability of the patients presented in this paper. It is reasonable to suppose that if the hematocrit varies in different parts of the body in health it will also vary in disease, in all probability to a much greater extent. Our observations indicate that total blood volume determined by the dye method alone compared with the total blood volume determined by the radioactive red cell method alone shows the total blood volume by radioactive red cells to be only about 81 per cent of the total blood volume determined by blue dye. This is in good agreement with Gibson's findings.

It is evident that a discrepancy exists between radioactive red cell data and carbon monoxide data in regard to blood volume (9). It would be interesting and valuable to compare the methods simultaneously but we have not had the opportunity to do so.

Cruikshank and Whitfield (10) reported experiments which indicated that mixing following the injection of blue dye was probably complete in  $1\frac{1}{2}$  minutes. They explained the mixing phase of the plasma dye curve as being due to phagocytosis of the Evans blue by the reticulo-endothelial cells. If this premise is correct, regardless of whether one knows the amount of dye injected, an unknown fraction is immediately removed from the circulation, therefore leaving an also unknown fraction in circulation to become tied up to the plasma albumin. The plasma volume is calculated by dividing the total amount of dye by the concentration in the plasma after mixing has been accomplished. If we do not know accurately the amount of dye which is available and distributed in the plasma, no reasonable determination of the plasma volume is possible.

H. P. Smith (11) and more recently Cruikshank and Whitfield (10) showed that if one blocks the reticulo-endothelial system by a previous injection of dye or india ink there is no sharp drop in concentration following the second injection which would correspond to the "mixing-phase" of the dye curve. We feel that the work referred to above makes the validity of the blue dye method as a means of determining plasma volume open to question. Until the procedure can be reliably standardized, it is futile to attempt comparison of plasma volumes obtained by dye dilution procedures with those obtained indirectly following the determination of red cell volume, either with the radioactively tagged red cells or carbon monoxide method.

It is well known that the blue dye which remains in circulation a few hours following injection is tied to serum albumin (12). With this in mind, we injected one dog with a massive dose of Evans blue (180 mgm.) and allowed 24 hours to elapse. Twenty milliliters of this dog's citrated plasma was then injected into each of two recipient dogs. The "mixing phase", although not entirely absent, appeared definitely less marked than is usually encountered following the direct administration of dye alone. Such a technique, which essentially resembles the tagged cell method for red cell mass determination may be useful in settling some of the moot points in connection with plasma volume methods. At least, one is forced to rely on some such procedure or a blockage of the reticulo-endo-

thelial system in order to determine the one important unknown factor, namely the fraction of dye which is actually distributed in the circulation.

The linearity of the "disappearance" phase of the curve would suggest that it is not related to the concentration of the dye in the serum per se, but rather to the concentration of the material to which the dye is bound, probably the serum albumin. It is quite likely that this rate of disappearance in turn can be related to the turnover of serum albumin and in itself provide an interesting subject for study.

As Cruikshank and Whitfield (10) pointed out, there probably is no rationale involved in the use of the square root of the time as an abscissa in the study of the mixing and disappearance curve as originally suggested by King, Cole and Oppenheimer (13). As pointed out by Hahn, Ross, Bale, Balfour and Whipple (3) there are probably at least three phases to the curve, and further attention to each individual phase should be considered in the light of the recently reported work.

#### SUMMARY AND CONCLUSIONS

The application of radioactive red cells to determination of human red cell mass is described and compared with simultaneous blue dye determinations. The previously reported discrepancy between findings by dye methods and direct cell methods is confirmed. In a group of 28 male patients in a variety of clinical states, great variability in the discrepancy between the two methods was observed. Until methods are more fully worked out, great caution should be exercised in estimating plasma volume or cell volume indirectly.

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# SODIUM THERAPY OF EXPERIMENTAL TOURNIQUET SHOCK<sup>1</sup>

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The accepted treatment of shock during World War II has emphasized replacement of fluids lost from the vascular system of the shocked patient.

Such replacement should be performed promptly before the so-called irreversible stage of shock has ensued; it should be of a qualitative nature chosen to fit the needs of replacement, and it should be of a quantity commensurate with the lost volume. This treatment is still the standard therapy of shock and these rules apply to the management of shock of several varieties including burn shock, traumatic shock, hemorrhagic shock, tourniquet shock, and others, both clinical and experimental. This method of therapy of shock is based on the original observations of Blalock (5) and of Parsons and Phemister (29). These authors showed that in experimental shock in animals sufficient local loss of blood elements occurs from the blood stream to indicate replacement therapy. This work led to the establishment of the entire war program of blood and plasma banks.

More latterly there has appeared a series of at least 35 articles (1-4, 7-14, 16-28, 30-33, 35-40) published since 1938 emphasizing the importance of sodium salts in the therapy of shock. Some writers (Hoitink, 17, Allen, 1-4, Rosenthal, 31, 32, and Fox 13, 14) go so far as to claim possible advantages for sodium therapy as compared to plasma administration. The work of Rosenthal on mice is well controlled but the comparative aspect has not been extended to larger animals. Fox's observations are largely clinical while Allen gives few comparative statistical details although his experimental protocols show good results with saline. Hoitink's conclusions are, on the other hand, entirely theoretical. This Dutch author stated in his widely quoted article (1938) that "0.9 per cent sodium chloride solution after an acute dangerous hemorrhage is preferable to the use of other artificial substitutes and to blood transfusion." Actually this author reported no experiments comparing sodium chloride solution with whole blood (or with plasma either for that matter) and based his preference on theoretical grounds, citing obsolete impressions that "great dangers connected with blood transfusion have influenced me." Hoitink in his experiments merely compared the efficacy of normal salt solution with other saline mixtures such as Tyrode's and Ringer's solutions. Scott, Worth and Robbins (33) found saline solution to be as effective as plasma in the treatment of venous tourniquet shock in dogs.

<sup>1</sup> The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Johns Hopkins University.

Some writers have merely discussed various aspects of the use of saline therapy, while still others have pointed out that their studies indicate such treatment to be inferior to plasma infusions in the treatment of shock. Thus Katz, Friedberg and Asher (19) found that saline is of value and is of greater efficacy than is glucose, but they make no comparison of its action with that of plasma or whole blood. Mylon and Winternitz (1946) reported that "sodium pretreatment of the dog in contrast to the mouse is followed by only slight tendency to longer survival." Mylon, Winternitz and De Sütö-Nagy (26) found saline to be inferior to plasma in the treatment of tourniquet shock in dogs. McKee, Laycock, Martens and Nicholl (22) studied hemorrhagic shock in dogs and found saline infusions to be far inferior to plasma therapy.

A perusal of the literature thus shows that while many articles have been written on saline therapy of shock, few have shown any evidence for the superiority of saline over plasma. What evidence there is has been chiefly presented in theoretical or unprotocoled papers, while definitive experiments have been limited to small animals (mice). That saline is relatively efficacious under certain conditions seems, however, to be well established.

No reports in the literature have shown saline to be superior to whole blood in the treatment of shock. Rosenthal (31) reported that saline by mouth (8 per cent of body weight) is as efficacious as whole blood by vein (3 per cent body weight) in the treatment of hemorrhagic shock, but that in equal quantities "whole blood is superior to saline or serum."

The present paper outlines certain experiments concerning the use of sodium lactate as a therapeutic agent in the management of tourniquet shock in rats. In-so-far as possible, previous experimental conditions reported by Rosenthal (31) on mice were duplicated on rats. This was done because Rosenthal's experiments serve as one of the most extensive and best documented pieces of evidence for the value of sodium salts in shock and because it was thought advisable to test his results on another species of animal.

**EXPERIMENTAL TECHNIC.** Three hundred and fifty-six male albino rats of the Sprague-Dawley strain were used in these experiments. The rats were placed in individual cages without food but with water *ad libitum* in the afternoon 14 to 16 hours before the start of the experiment. The next morning the water was removed and the rats were weighed and found to vary between 210 and 261 grams. The animals were then anesthetized with ether and rubber band tourniquets were applied high on both thighs by the method of Rosenthal (31). Uniform bands (Eberhard Farber, no. 30) were twisted five turns on a section of glass tubing 4 inches long with an inside diameter of one inch. A ten gram weight was attached by a 6 inch piece of heavy braided silk to the ankles of the rats to facilitate putting the glass tube over the thigh. The tubing was then pushed high on the thigh after moistening it with water (to facilitate removal of the rubber bands), at the same time holding the leg straight with the braided silk and with the genitalia out of the way. The band was then shoved off the tubing onto the flesh and the procedure was repeated for the other leg. In the last 29 rats, the tips of the four incisor teeth were cut off with heavy scissors while the animals were still anesthetized.

The rats were then replaced in the individual cages. The tourniquets were now left in place for exactly three hours, at the end of which time they were cut off. Because of anesthesia of the limbs, a number of the animals bit their legs during the period the tourniquets were in place. Because of the resultant bleeding, these animals were discarded, and of the 356 rats, 89 were so discarded. Bites did not occur when the incisor teeth were cut off. In all instances the discarding was done at once at the start of the experiment rather than at the end when if selectively done it would influence the mortality percentage. In addition, 12 rats were not counted because the room temperature was above 26.7°C. All the 254 other rats were tested with the room temperature between 21.0°C. and 26.7°C. during the entire 53 hour period after the application of the tourniquets.

At the time of removal of the tourniquets, the rats were given one dose of the test medication (usually 10 cc., i.e., approximately 5 per cent body weight, of the solution, usually by stomach tube—a two inch portion of small ureteral or urethral catheter on the end of a 20 gauge needle attached to a 10 cc. Luer-Lok syringe containing the solution). The animals were then replaced in the individual cages and three hours later a second dose of the medication was given. The rats were then observed for 50 hours after removal of the tourniquets, at which time, if still alive, they were sacrificed. The time of survival, when less than 50 hours, was recorded as the time till death after removal of the tourniquets.

In all instances marked swelling of the hind limbs was noted. The lungs showed no evidences of aspiration of the fluid given.

**RESULTS.** *Experiment 1. Controls.* Forty-six rats were used as no-therapy controls, being allowed to continue to exist without treatment, water, or food from the time of removal of the tourniquets until death, or until a maximum period of 50 hours had elapsed. As seen in the last two columns of table 1, 80 per cent of these animals died during the first 24 hours and 93 per cent were dead at the end of 50 hours.

*Experiment 2. Water per os.* In the second series, 38 rats were given water by the stomach tube (10 cc. immediately after release of the tourniquets and 10 cc. 3 hrs. later, a total of 20 cc. or approximately 10 per cent of the body weight). This treatment prolonged the lives of the animals considerably as evidenced by the figures in table 1. At the end of 24 hours the mortality was only slightly more than half that of the control series and at the end of 50 hours almost five times as many animals were alive. Table 2 indicates that the beneficial effect of water is not only demonstrated by the entire group of animals as a whole, but by the individual experiments.

*Experiment 3. Molar sodium lactate per os.* This solution was administered to 46 rats in amounts so that the animals received exactly as much sodium lactate as those in experiment 4 below, but of necessity only one-sixth as much water. As seen in table 1, the 24 hour and total 50 hour mortality rates were definitely lower than those of the no-therapy controls in experiment 1, but were slightly higher than those following water alone in experiment 2.

*Experiment 4. One-sixth molar sodium lactate per os.* This solution was

given in full doses (10 cc. instead of 1.8 cc. as for molar lactate in expt. 3). The 24 hour mortality of 16 per cent and the total 50 hour mortality of 37 per cent represented by far the best results obtained in this series of experiments. Actually, as seen from the recovery columns in table 1, at the end of 50 hours, as based on percentage of recovery, nine times as many rats were alive following one-sixth molar sodium lactate as in the control series, twice as many as following water therapy, and three times as many as following molar sodium lactate therapy. Table 2 shows that the relative merits of one-sixth molar sodium lactate in comparison with the effects of no treatment or of water, are demonstrated by every single one of 9 separate experiments, despite the fact that they were performed over a period covering more than 12 months. Furthermore,

TABLE 1  
*Mortality following electrolyte therapy in 255 rats with tourniquet shock*

EX- PER- IMENT NO.	THERAPY IMMEDIATELY AFTER AND 3 HOURS AFTER REMOVAL OF TOURNIQUETS	TOTAL NO. RATS	MORTALITY DETAIL												RECOVERY		MORTALITY SUMMARY	
			0-3 hrs.		3-24 hrs.		Total 0-24 hrs.		24-44 hrs.		44-50 hrs.		Total 24-50 hrs.		> 50 hrs.			
			No. Rats	Mort.	No. Rats	Mort.	No. Rats	Mort.	No. Rats	Mort.	No. Rats	Mort.	No. Rats	Mort.	No. Rats	Recov.		
1	None	46	2	4	35	76	37	80	6	13	0	0	6	13	3	7	80	93
2	10 ml. water per os	38	3	8	16	42	19	50	7	18	0	0	7	18	12	32	50	68
3	1.8 ml M Sodium Lactate per os	46	0	0	25	54	25	54	12	26	0	0	12	26	9	20	54	80
4	10 ml M/6 Sodium Lactate per os	86	0	0	14	16	14	16	17	20	1	1	18	21	54	63	16	37
5	10 ml M/6 Sodium Lactate i. p.	12	1	8	9	75	10	83	0	0	0	0	0	0	2	17	83	83
6a	10 ml 1.75% MgSO <sub>4</sub> i. p.	7	1	14	5	72	6	86	1	14	0	0	1	14	0	0	86	100
b	10 ml 1.75 MgSO <sub>4</sub> per os	3	0	0	1	33	1	33	1	33	0	0	1	33	1	33	33	66
7a	4 ml 1% KCl i. p.	9	1	11	7	78	8	89	0	0	0	0	0	0	1	11	89	89
b	4 ml 1% KCl i. p. + 10 ml M/6 Sodium Lac- tate per os	8	1	13	3	37	4	50	0	0	0	0	0	0	4	50	50	50
Totals and averages.....		255	9	4	115	45	124	48	44	17	1	0	45	18	86	34	48	66

table 3 demonstrates that one-sixth molar sodium lactate was superior to molar sodium lactate in 7 out of 8 other separate experiments covering a period of over 12 months.

*Experiment 5. One-sixth molar sodium lactate intraperitoneally.* The solution was given to 12 rats intraperitoneally in the same doses as were used in experiment 4 by the oral route. The results were far poorer, and as seen in the last columns of table 1, the mortality approximated that in the control series, being slightly worse for the first 24 hours and slightly better for the entire period. This slight difference in the time of mortality may possibly be explained by slow absorption of the peritoneal fluid in the presence of shock.

*Experiment 6. (a) One and seventy-five hundredths per cent MgSO<sub>4</sub> intraperi-*

toneally. This solution was given to 7 rats intraperitoneally in volumes equal to those used for one-sixth molar sodium lactate solution in experiments 3 and 5. The 24 hour mortalities were about the same as following one-sixth molar sodium lactate solution intraperitoneally, but no animals lived beyond 50 hours.

(b) One and seventy-five hundredths per cent  $MgSO_4$  per os. In a scanning

TABLE 2

Individual experiments each on the same day comparing no-treatment-control rats, water-by-mouth rats, and one-sixth-molar-sodium-lactate-by-mouth rats. (In each case the numerator of the fraction denotes the number of rats dead within 24 hours and the denominator denotes the total number of rats tested in that individual experiment.)

EXPERIMENT.....	A	B	C	D	E	F	G	H	I	MEAN*	Column 1 vs 2		Column 1 vs 3		Column 2 vs 3	
	Mortality percentage										t	P†	t	P†	t	P†
										%						
1. No-treatment controls (total = 28 rats)	100 (3/3)	100 (5/5)	67 (2/3)	100 (4/4)	0 (0/1)	67 (2/3)	100 (2/2)	75 (3/4)	67 (2/3)	75 ± 11%	1.5	<0.2	4.7	<0.001		
2. H <sub>2</sub> O per os (total = 38 rats)	75 (3/4)	67 (4/6)	100 (3/3)	75 (3/4)	17 (1/6)	0 (0/3)	0 (0/3)	75 (3/4)	67 (2/3)	50 ± 13%	1.5	<0.2			2.6	0.02
3. M/6 sodium lactate per os (total = 31 rats)	0 (0/2)	20 (1/5)	17 (1/6)	33 (1/3)	0 (0/3)	0 (0/3)	0 (0/2)	33 (1/3)	50 (2/4)	17 ± 6%			4.7	<0.001	2.6	0.02

\* Differs from figure in table 1 because not all rats included here.

† Values below 0.05 are considered significant.

N.B. If the 50 hour mortalities are compared as in columns 1 and 2 for the no-treatment controls and for the H<sub>2</sub>O per os rats, P = 0.3. The combined P of the 24 and 50 hour mortalities is then 0.06 or 0.12 which is just above the borderline of statistical significance.

TABLE 3

Individual experiments each on the same day comparing one-sixth molar-lactate-by-mouth rats with molar-lactate-by-mouth rats. (In each case the numerator of the fraction denotes the number of rats dead within 24 hours and the denominator denotes the total number of rats tested in that individual experiment.)

EXPERIMENT .....	I	J	K	L	M	N	O	P	Mean*	t	P
	Mortality percentage										
M/6 sodium lactate per os (total = 42 rats)	0 (0/7)	14 (1/7)	20 (1/5)	0 (0/5)	0 (0/3)	67 (2/3)	0 (0/9)	0 (0/3)	13 ± 8		
Molarsodium lactate per os (total = 46 rats)	38 (3/8)	57 (4/7)	67 (4/6)	20 (1/5)	100 (4/4)	50 (1/2)	44 (4/9)	80 (4/5)	57 ± 8	3.7	0.01†

\* Differs from figure in table 1 because not all rats included here.

† Values below 0.05 are considered significant.

experiment 3 additional rats were given similar volumes of magnesium sulfate solution by mouth with slightly better recovery percentages, although obviously the number of animals studied is too small for accurate comparisons.

Experiment 7. (a) One per cent KCl intraperitoneally. Such a solution was given intraperitoneally to 9 rats, 4 cc. at the time of tourniquet release and 4 cc.

3 hours later. A high mortality of 89 per cent at the end of 24 hours resulted, but the total mortality was no worse than in the control series.

(b) *One per cent KCl intraperitoneally plus one-sixth molar sodium lactate per os.* In 8 other rats, similar amounts of 1 per cent potassium chloride solution were given intraperitoneally and at the same time oral dosages of one-sixth molar sodium lactate solution similar to those administered in experiment 4 were given. While the number of animals used is too small for conclusive assay, the mortality percentages of the KCl-Na lactate combination are just about half way between the high mortality of the KCl animals (expt. 7 a) and the low mortality of the one-sixth molar sodium lactate animals (expt. 4), as though the two solutions had neutralized each other's effects even though given by different routes. It is realized that several factors mitigate against the conclusiveness of the results in this experiment aside from the small number of animals tested, namely, the lack of KCl by mouth controls and of oral potassium chloride and of intraperitoneal water controls.

DISCUSSION. These experiments indicate that water exerts a moderate sparing action while one-sixth molar sodium lactate solution demonstrates a marked sparing action in shock in rats after tourniquet release. Such results on rats confirm those of Rosenthal on mice in this regard. Our own experiments made no comparison of the efficacy of sodium lactate with that of plasma or whole blood. In Rosenthal's experiments (31) on mice the tourniquets were looped six times instead of five as in our experiments and were left on two instead of three hours. The mortalities were quite similar in Rosenthal's and in our experiments, the control 48 hour mortalities being 95 and 93 per cent and the mortalities after sodium therapy 25 and 16 per cent respectively. (Rosenthal used sodium chloride, we used sodium lactate solution. In other experiments on tourniquet shock, burns, and after hemorrhage Rosenthal found sodium lactate and sodium chloride to be equally efficacious.)

Intraperitoneal sodium lactate solution seemed to be far less efficacious in our experiments than oral medication, the 24 hour mortalities being 83 and 16 per cent respectively. Rosenthal found the two routes to be equally efficacious after tourniquet shock and after burns.

In the present experiments water by mouth was found to be about one-half as useful as was one-sixth molar sodium lactate. The comparison between water and the no-therapy controls in our experiments was, however, as shown in table 2, barely statistically significant. Rosenthal found water to be of no value following burns but of slight usefulness after hemorrhage.

In our experiments the reduction in mortality produced by smaller amounts of oral concentrated (molar) sodium lactate solution was not nearly as marked as that produced by larger volumes of the dilute (one-sixth molar) solution by the same route, in each case the amount of sodium salt given being the same. As seen in table 3 these results had a highly significant statistical difference. Actually the molar lactate gave results about equal to that of water alone. This would indicate that both the water and the sodium salt in the dilute sodium lactate solution may be of value and not either alone. Rosenthal (31) found

that the same amount of sodium chloride given by mouth became less efficacious in the treatment of burn shock the less water it was mixed with.

Potassium salts given intraperitoneally were found to be toxic to tourniquet shocked rats although complete controls were not done for this experiment. However, the coincident administration of oral sodium lactate markedly reduced the mortality rate. Rosenthal (31) found a marked toxicity for burned mice resulting from the use of KCl by mouth and also noted that the potassium salts antagonize the action of NaCl.

Similarly, magnesium sulfate intraperitoneally in 1.75 per cent solution gave results comparable to the no-therapy controls. No definite conclusions can be drawn from this experiment because of the lack of controls for all aspects of it. However, the results are of interest in connection with the paper of Green and Stoner (15) on magnesium potentiation of shock.

In our experiments the effect of room temperature was quite marked. Because the deleterious effect of excessive heat in shock is well known, no complete data on this subject were prepared. However, the trend of results in a small series (10 rats at 29.5–30.6°C, mortality of 100 per cent in 24 hrs.; 6 rats at 21.8° to 24.7°C., mortality of 50 per cent in 24 hrs.) which was repeated in a larger number of unrecorded experiments is of interest. In our experiments no experiments were included with the room temperature above 26.7°C.

It is also of interest to note from table 1 that of the entire series of 255 rats, 123 (48 per cent) died during the first 24 hours, 45 (18 per cent) died between 24 and 50 hours, and 86 (33 per cent) lived beyond 50 hours. Furthermore, of the entire group, only one rat died between 44 and 50 hours, indicating that in the rat, if the animal has not succumbed from shock before that time, he is apt to survive, at least until the end of the 50 hour period.

In arriving at the standard technic of producing tourniquet shock (5 turns of the rubber band for 3 hours on both legs) with an 80 per cent 24 hour mortality several other combinations of numbers of turns and of time were tried. In a small series of rats the following 24 hour mortalities were obtained: 100 per cent (4 turns, 4 hrs.), 20 per cent (5 turns, 2 hrs. at high temperature), 0 per cent (5 turns, 2½ hrs.), 80 per cent (5 turns, 3 hrs.), 100 per cent (5 turns, 5 hrs.). The marked influence of the duration of application of the tourniquets in rats has also been shown to exist by Shipley, Meyer, and McShan (34).

The results of the experiments indicate that both water and sodium salts are beneficial to rats with shock due to tourniquet shock. Such a benefit is probably due to disturbed fluid and salt balance. However, this does not mean that at the same time whole blood or plasma therapy may not be also or additively efficacious; nor does it mean that because there is an altered fluid and salt balance, there is not likewise a loss of colloidal blood elements from the blood stream.

#### CONCLUSIONS

1. With a standardized technic of production of tourniquet shock in etherized rats, water given by mouth after the release of the tourniquets exerts a sparing action as compared to no-treatment control animals.

2. In similar experiments, one-sixth molar sodium lactate solution by mouth exerts a far more striking sparing action. This beneficial effect is not exhibited when equal amounts of one-sixth molar sodium lactate solution are given intraperitoneally.

3. Oral sodium lactate in more concentrated form (molar solutions with equal amounts of sodium lactate but only one-sixth as much water) exerts a very moderate sparing action about the same as or poorer than that produced by water alone. Thus, the 24 hour mortalities are: controls (80 per cent), water (50 per cent), molar sodium lactate (54 per cent), and one-sixth molar sodium lactate (16 per cent). The respective total 50 hour mortalities are 93 per cent, 68 per cent, 80 per cent, and 37 per cent.

4. The preceding conclusions would lead to the further premise that under these conditions and in these animals, the beneficial effects of one-sixth molar sodium lactate may be due almost equally to the contained sodium salts and to the water, and not to either solute or solvent alone.

5. While these results indicate a definite therapeutic value for sodium lactate and for water in tourniquet shock in the rat, they do not attempt to compare the merits of such agents with the clinically proven usefulness of plasma or whole blood transfusions. Furthermore, a search of the literature reveals no instance of experimental proof that sodium salts are superior to whole blood transfusions in the treatment of any type of shock.

Mr. Stuart R. Elliott, II, gave valuable technical assistance.

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# THE IMPORTANCE OF THE AFFERENT NERVOUS FACTOR IN EXPERIMENTAL TRAUMATIC SHOCK: THE EFFECT OF CHRONIC DEAFFERENTATION<sup>1, 2</sup>

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Many workers have attempted to show the presence of an afferent nervous factor in experimental traumatic shock. The problem has been investigated by interrupting the pathways which carry nervous impulses from the region of injury. Blocking of nervous impulses has been achieved by *a*, section of peripheral nerves (1, 2, 3); *b*, local anesthesia (3, 4, 5); *c*, partial or complete transection of the spinal cord (2, 3, 5, 6), and *d*, spinal anesthesia (2, 4, 7, 8). There has been considerable disagreement in the interpretation of the results of such studies, for no one has advanced convincing evidence of a neurogenic factor in traumatic shock. It has been difficult not only to evaluate the severity of injury, but also to ascertain the importance of the effects which are produced by the above procedures in addition to those concerned with interruption of the afferent pathways.

In a recent communication (9) it has been shown that animals receiving muscle trauma are unable to withstand as much blood loss as hemorrhaged animals. Indeed, traumatized animals have a 50 per cent mortality if the circulating blood volume is reduced to 73.4 cc. per kgm. of body weight, whereas in the hemorrhage series the same mortality rate prevails at a residual volume of 59.1 cc. per kgm. of body weight. If this difference is the result of the action of afferent impulses from the injured region, the mortality rate following muscle trauma should be lowered when afferent pathways are interrupted. In the present report, this hypothesis has been tested on dogs in which the dorsal roots from both hind legs have been sectioned. Their responses to muscle trauma have been compared with those obtained on traumatized normal dogs reported by Wang et al. (9).

**METHODS.** Thirty muscle trauma experiments were performed on healthy mongrels in which the hind legs were previously deafferented. In 18 of these animals, a blood volume determination was made on the day before the operation was scheduled (10, 11). The operation was performed aseptically under intravenous nembutal anesthesia (pentobarbital sodium, 35 mgm. per kgm. of body weight). The dorsal surface of the lumbar spinal cord was exposed through a laminectomy wound. All 7 lumbar dorsal roots on both sides and the three sacral roots on one side were sectioned intradurally. The 3 sacral roots on the

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<sup>2</sup> A preliminary report of this work appeared in *Fed. Proc.* 5: 110, 1946.

opposite side were left intact in order to preserve the function of micturition. The dura was approximated and the wound closed. There was no operative mortality, but the post-operative course of the animals was variable. Most of the dogs showed a loss of weight during the first week after operation. They were able to stand and walk, the hind limbs showing considerable ataxia. A large number of the animals developed cage sores, usually on the side on which both the lumbar and sacral dorsal roots had been cut. In only a few instances did the ulceration become so extensive that the animals were unfit for muscle trauma experiments. The interval between the operation and the trauma experiment depended upon the condition of the hind legs and the general health of the animals and varied from 14 to 58 days, the average being 30 days. At the time of the muscle trauma experiments, almost all the animals showed either a slight gain in weight or the loss was within 10 per cent of the pre-operative weight. Although the weight loss following operation exceeded 10 per cent in dogs D7, D14, D18 and D24, these animals were used because they had a good appetite and appeared to be in good health.

The procedure used in the trauma experiments has been given in detail in another publication (9). On the day before the trauma experiment, a control plasma volume determination was carried out, using the dye (T-1824) dilution method (10, 11). The total blood volume was calculated from the measured plasma volume and the hematocrit value. The serum protein concentration was determined refractometrically (12). On the following day control determinations of heart rate, mean blood pressure (by femoral arterial puncture) and rectal temperature were made. Ether was then given and the muscles of both thighs were contused (13). The thigh in which both the lumbar and sacral dorsal roots were cut was contused first so as to receive most of the swelling and injury. After trauma the animal was put back on the board and the administration of ether was discontinued. The femoral artery and vein were exposed under 2 per cent procaine anesthesia. Thereafter, determinations of the heart rate, mean blood pressure and rectal temperature were repeated at regular intervals. Another determination of the circulating blood volume was made one to two hours after muscle trauma. After injury, the animal was kept on the board for a period of 6 hours or until death occurred. If the animal survived beyond the 6th hour, the wound in the femoral region was closed, and the dog was put into a cage and given water. Animals which were alive 24 hours later were counted as having survived.

**RESULTS.** The values for blood volume, hematocrit reading and serum protein concentration determined before section of the lumbo-sacral dorsal roots and again before muscle trauma are shown in table 1. The data obtained from 18 dogs in which such controls were carried out indicate that on the average there was no change in the serum protein concentrations which remained at 6 grams per cent. The slight (statistically not significant) reduction in blood volume (from 100.3 to 98.2 cc. per kgm., a decrease of 2.1 cc. per kgm.) can be accounted for by the small decrease in hematocrit values (from 44.6 to 42.3 per cent, a decrease of 2.3 per cent). The change in body weight which can be taken as an

indication of the general condition of the animal was also small (decreasing from an average of 10.7 kgm. to 10.3 kgm., see table 2). Even in the few dogs in which the values obtained before deafferentation differed significantly from those measured before injury, the changes observed did not appear to have favored the survival of the animal. On account of the intact sacral roots on one side, all the animals retained the ability of spontaneous micturition, although a number showed some degree of urinary retention, especially during the early days following the dorsal root section. It was not necessary to express the urine

TABLE 1

*Comparison of blood volume, hematocrit value and serum protein concentration before and after deafferentation*

DOG NUMBER AND SEX	AT TIME OF OPERATION			BEFORE TRAUMA EXPT.			CHANGE			FATE
	B. V.	Hct.	Protein	B. V.	Hct.	Protein	B. V.	Hct.	Protein	
	cc./kgm.	per cent	gm. %	cc./kgm.	per cent	gm. %	cc./kgm.	per cent	gm. %	
D5 ♀	96	38.0	6.3	97	38.4	6.1	+1	+0.4	-0.2	Survived
D6 ♀	99	42.8	5.9	93	37.8	6.2	-6	-5.0	+0.3	Survived
D7 ♀	106	46.5	5.8	102	43.9	5.3	-4	-2.6	-0.5	Survived
D8 ♂	99	36.5	4.7	102	36.0	6.1	+3	-0.5	+1.4	Survived
D9 ♂	103	39.2	5.0	103	38.1	4.8	0	-1.1	-0.2	Survived
D10 ♀	93	36.6	6.1	94	42.9	6.1	+1	+6.3	0	Survived
D12 ♂	104	45.8	6.2	104	50.1	6.5	0	+4.3	+0.3	Survived
D13 ♂	100	52.8	7.1	118	48.1	7.0	+18	-4.7	-0.1	Died
D14 ♂	99	48.2	5.9	96	45.3	6.5	-3	-2.9	+0.6	Died
D18 ♀	104	40.5	6.2	94	31.1	5.7	-10	-9.4	-0.5	Survived
D19 ♀	108	49.9	5.6	95	47.2	6.5	-13	-2.7	+0.9	Survived
D22 ♀	86	45.9	6.2	93	43.6	6.2	+7	-2.3	0	Survived
D23 ♂	106	36.1	6.5	102	40.8	5.9	-4	+4.7	-0.6	Died
D24 ♀	104	50.5	5.6	113	42.3	6.1	+9	-8.2	+0.5	Died
D25 ♂	96	54.4	6.1	87	50.3	6.6	-9	-4.1	+0.5	Died
D28 ♀	108	52.2	5.4	98	41.6	6.0	-10	-10.6	+0.6	Died
D29 ♀	96	42.8	6.2	88	45.8	5.4	-8	+3.0	-0.8	Died
D30 ♂	98	44.3	6.4	88	37.9	5.2	-10	-6.4	-1.2	Died
Mean..	100.3	44.6	6.0	98.2	42.3	6.0	-2.1	-2.3	0	

manually as in spinal animals. The retention, when present, improved slowly and in most cases the bladder was not distended at the time of trauma experiments. No gross atrophy of the deafferented limb muscles was observed.

The results of the trauma experiments are shown in table 2. The animals have been arranged according to their residual blood volumes, that is, the blood volume present after muscle injury expressed in cc. per kgm. of body weight. It is apparent that an animal has a fair chance of survival if the residual blood volume is not reduced below 65 cc. per kgm. In a single animal (D2) that died with the relatively high residual volume of 76 cc. per kgm., there was evidence

TABLE 2

*Data on chronic hind leg deafferented dogs before and after muscle trauma, including changes in blood volumes, hematocrit values and serum protein concentrations*

DOG NUMBER AND SEX	BODY WEIGHT IN KG.M.	CONTROL			AFTER MUSCLE TRAUMA			CHANGE			FATE
		B. V.	Hct.	Protein	B. V.	Hct.	Protein	B. V.	Hct.	Protein	
		(1)	(2)	(3)	(4)	(5)	(6)	$\frac{(1)-(4)}{100} \times$	(5)-(2)	(6)-(3)	
		cc./ kgm.	per cent	gm. %	cc./ kgm.	per cent	gm. %	per cent	per cent	gm. %	
D1 ♂	10.6(11.3*)	97	36.8	5.5	76	43.9	5.4	22	+7.1	-0.1	Survived
D2 ♂	10.3( 9.0)	100	41.4	6.3	76	47.9	5.8	24	+6.5	-0.5	Died†, 6+ hr.
D3 ♂	10.1(10.3)	115	35.9	7.1	74	31.0	6.9	36	-4.9	-0.2	Survived
D4 ♀	7.9( 8.6)	96	34.6	5.5	73	36.3	5.1	24	+1.7	-0.4	Survived
D5 ♀	11.8(11.9)	97	38.4	6.1	73	46.3	5.5	25	+7.9	-0.6	Survived
D6 ♀	7.5( 7.6)	93	37.8	6.2	71	40.7	5.4	24	+2.9	-0.8	Survived
D7 ♀	7.4( 8.7)	102	43.9	5.3	71	41.1	4.7	30	-2.8	-0.6	Survived
D8 ♂	9.5( 9.2)	102	36.0	6.1	70	33.9	5.3	31	-2.1	-0.8	Survived
D9 ♂	8.1( 8.1)	103	38.1	4.8	70	39.6	4.1	32	+1.5	-0.7	Survived
D10 ♀	11.1(10.7)	94	42.9	6.1	69	48.8	6.1	27	+5.9	0	Survived
D11 ♂	8.0( 7.7)	105	36.4	7.8	69	37.9	7.0	34	+1.5	-0.8	Survived
D12 ♂	13.3(14.3)	104	50.1	6.5	69	55.5	6.3	34	+5.4	-0.2	Survived
D13 ♂	13.2(14.5)	118	48.1	7.0	69	47.4	6.6	42	-0.7	-0.4	Died, 3.4 hr.
D14 ♂	14.0(16.2)	96	45.3	6.5	67	47.1	6.5	30	+1.8	0	Died, 4.9 hr.
D15 ♂	7.8( 8.5)	107	38.6	7.0	67	34.8	6.4	37	-3.8	-0.6	Died, 3 hr.
D16 ♂	7.6( 8.5)	90	40.3	5.2	66	40.0	5.0	27	-0.3	-0.2	Survived
D17 ♀	10.0(10.0)	91	35.2	6.3	66	36.1	5.9	27	+0.9	-0.4	Survived
D18 ♀	10.1(11.5)	94	31.1	5.7	65	37.4	5.2	31	+6.3	-0.5	Survived
D19 ♀	10.0( 8.9)	95	47.2	6.5	65	48.8	5.8	32	+1.6	-0.7	Survived
D20 ♀	14.4(14.7)	111	29.8	7.2	65	30.7	6.5	41	+0.9	-0.7	Died, 3.9 hr.
D21 ♀	9.9( 9.6)	99	36.1	6.8	64	37.7	6.3	35	+1.6	-0.5	Died, 6+ hr.
D22 ♀	10.4(10.8)	93	43.6	6.2	63	48.6	5.6	32	+5.0	-0.6	Survived
D23 ♂	11.1(10.7)	102	40.8	5.9	62	40.6	5.2	39	-0.2	-0.7	Died, 3.8 hr.
D24 ♀	10.5(12.3)	113	42.3	6.1	62	39.3	5.6	45	-3.0	-0.5	Died, 3 hr.
D25 ♂	15.0(15.5)	87	50.3	6.6	61	56.3	6.1	30	+6.0	-0.5	Died, 6+ hr.
D26 ♀	9.0( 9.5)	109	48.7	5.8	60	43.3	5.0	45	-5.4	-0.8	Died, 3.4 hr.
D27 ♂	9.0( 9.5)	87	38.7	5.2	59	49.4	5.0	32	+10.7	-0.2	Survived
D28 ♀	9.6( 9.3)	98	41.6	6.0	59	42.0	6.2	40	+0.4	+0.2	Died, 6 hr.
D29 ♀	12.0(12.0)	88	45.8	5.4	58	44.5	4.6	34	-1.3	-0.8	Died, 4 hr.
D30 ♂	9.6(10.4)	88	37.9	5.2	57	38.9	4.4	35	+1.0	-0.8	Died, 3.9 hr.
Mean.....	10.3(10.7)	99.1	40.5	6.1		42.2	5.6		+1.7	-0.5	
Standard error....		1.5	0.9	0.1					0.7	0.05	

\* Body weight at the time of deafferentation.

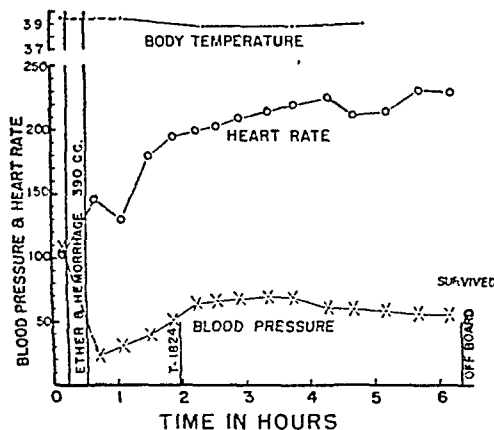
† Both hind legs, particularly the perineal region, sensitive to pain.

that the injured regions were less completely deafferented than was the case in the other animals of this series (see discussion).

The mortality rate of the entire series of 30 animals with residual volumes varying between 57 and 76 cc. per kgm. was 43 per cent. A further statistical analysis of the data by the method suggested by Doctor Fertig (see 9) shows that the residual blood volume at the 50 per cent mortality point is  $64.7 \pm 1.8$  cc. per kgm. of body weight. The percentage survival at a residual volume of 66 cc. per kgm., which is the value previously used in comparing various series of animals (see 9), is  $59 \pm 10.8$  per cent. The average change of the hematocrit values was an increase of  $1.7 \pm 0.7$  per cent (from 40.5 to 42.2 per cent) and that of

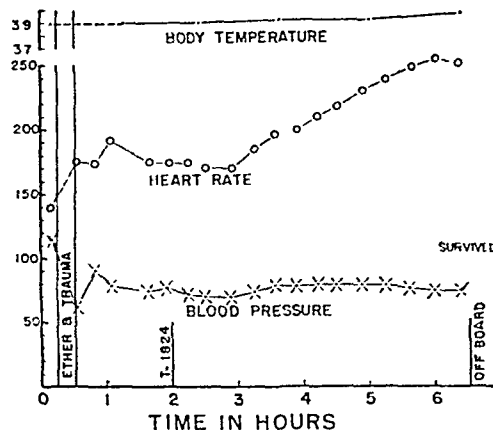
### HEMORRHAGE NORMAL DOG H13# WEIGHT 12.3 KGM.

	BEFORE	AFTER
BLOOD VOLUME, cc./kgm.	90	62
HEMATOCRIT, %	33	26
SERUM PROTEIN, gm. %	4.9	4.2



### TRAUMA DOG D22# WEIGHT 10.3 KGM WITH DORSAL ROOTS CUT

	BEFORE	AFTER
BLOOD VOLUME, cc./kgm.	93	63
HEMATOCRIT, %	44	49
SERUM PROTEIN, gm. %	6.2	5.6



### TRAUMA NORMAL DOG T31# WEIGHT 10.7 KGM

	BEFORE	AFTER
BLOOD VOLUME, cc./kgm.	93	62
HEMATOCRIT, %	38	52
SERUM PROTEIN, gm. %	5.5	6.3

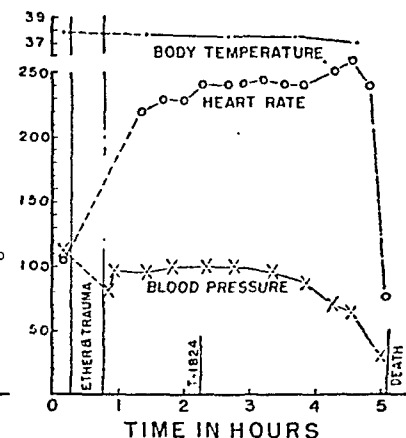


Fig. 1. Comparison of the clinical manifestations (body temperature, heart rate and mean blood pressure) in a deafferented animal which has been subjected to muscle trauma (D22) with those in a typical hemorrhaged (H13) and those in a typical traumatized animal (T31). Note the slowly increasing tachycardia of dogs D22 and H13. The mean blood pressure of D22 immediately following muscle trauma is below 70 mm. Hg and its plateau pressure is also lower than that of dog T31. Like dog H13, dog D22 survived the injury. The experiments on dogs H13 and T31 are taken from a paper by Wang et al. (9).

serum protein concentrations, a decrease of  $0.5 \pm 0.05$  gram per cent (from 6.1 to 5.6 gram per cent).

The clinical signs of the traumatized, deafferented dogs are different from those shown by normal dogs receiving muscle trauma. In figure 1 the clinical responses to blood volume reduction are shown in 3 different types of preparations: a, H13, simple hemorrhage; b, D22, chronic deafferentation with muscle injury; and c, T31, muscle trauma. It should be noted that although these three animals have approximately the same control blood volumes (90 to 93 cc. per kgm.) and about the same residual volumes (62 to 63 cc. per kgm.), the deafferented animal, like the simple hemorrhaged animal, survived the injury, whereas the normal traumatized dog succumbed. The deafferented dog (D22) demonstrated other similarities as well as certain discrepancies when compared with the

simple hemorrhaged and the traumatized normal animals, respectively. The three typical preparations had a tachycardia exceeding 200 beats per minute. In both D22 and H13, 2 to 3 hours were required to reach this rapid rate, while dog T31 developed the rapid rate immediately after the injury. Dog D22 had an initial mean blood pressure of 62 mm. Hg following injury and survived even though its mean blood pressure was maintained as low as 70 to 80 mm. Hg. Many of the deafferented animals, which succumbed to muscle injury, had a plateau level of mean blood pressure less than 70 mm. Hg for a number of hours. As a group, the deafferented animals were quiet, and showed no depression of the central nervous system until a short time before death.

**DISCUSSION.** In 1930 Blalock (14) and Parsons and Phemister (1) observed that the local fluid loss which results from severe trauma to the thigh muscles extends upward into the flank and showed that there is a large difference in weight between the normal and the traumatized sides. This indicates merely that the loss of circulating fluid into the local injured area constitutes one of the most important changes occurring in traumatic shock. These experiments do not, however, permit one to conclude that the fluid loss itself is sufficient to cause the death of all animals suffering traumatic injury. Recently Wang et al. (9) have studied dogs in which the blood volumes have been decreased by simple hemorrhage or by muscle trauma. They have reported a striking difference in the ability of these two groups of animals to withstand a specific loss of blood. In dogs in which the blood volume is reduced to 66 cc. per kgm. by simple hemorrhage the percentage survival is  $76 \pm 8.7$  per cent; if, on the other hand, a similar reduction is effected by muscle trauma the chance of survival is only  $25 \pm 8.3$  per cent. Hemorrhaged animals have a 50 per cent chance of survival if the blood volume is reduced to  $59.1 \pm 2.9$  cc. per kgm., whereas in muscle trauma the same chance of survival prevails when the residual volume is  $73.4 \pm 3.0$  cc. per kgm. From this, it is concluded that reduction of circulating blood volume alone is not adequate to explain the high mortality rate in traumatized dogs. If the afferent impulses from the injured region exert a deleterious effect, the exclusion of such impulses should enable the traumatized dogs to withstand a greater reduction of blood volume than if the afferent impulses had not been interrupted.

The simplest method in interrupting the afferent pathways is to apply procaine intrathecally or in the vicinity of the peripheral nerve trunks. However, most investigators have used also general anesthesia, which among other effects is known to modify reflex activities. Swingle and his associates (3, 4) have studied the effect of exclusion of afferent impulses in traumatic shock and have reported that many deaths are prevented by local or spinal procainization. Wang et al. (5) have repeated these experiments with blood volume determinations and observed that the chances of survival for these procaine treated animals are not enhanced when the blood volume is reduced to the same extent as in the traumatized animals which do not receive procaine. It is difficult to give procaine in analgesic doses without obtaining side effects on the neuro-circulatory system. In another series of experiments (5) it has been found that the death following muscle trauma is not prevented in chronic spinal preparations (transection at the

lower thoracic region). Since these spinal animals are not able to micturate spontaneously and are prone to develop cage sores, most of the trauma experiments have been carried out in the first post-operative week during which the animals have not fully recovered from the operation, as shown by a decline of body weight and a relatively low control blood volume.

It is true, of course, that the operation on the lumbo-sacral roots is much more extensive than simple transection of the spinal cord. However, the former has certain definite advantages in that such animals are deprived only of the afferent nerves, the mechanism for spontaneous micturition being preserved when some of the sacral roots were left intact. Although decubitus ulcers developed, these had usually healed at the time of the trauma experiment. The general condition of the animal was good, as indicated by the body weights and blood volumes not very different from their respective control determinations.

In the series of deafferented animals the over-all mortality rate is only 43 per cent, whereas in a series of 40 traumatized normal animals the mortality is 63 per cent (9). The residual blood volume at the 50 per cent mortality point in the deafferented series is  $64.7 \pm 1.8$  cc. per kgm. The difference between this and the corresponding value in the normal trauma series ( $73.4 \pm 3.0$  cc. per kgm., see 9) is statistically significant. When compared with the corresponding value of the hemorrhage series ( $59.1 \pm 2.9$  cc. per kgm.), the difference is not significant. Also, The percentage survival at a residual volume of 66 cc. per kgm. in the deafferented series ( $59 \pm 10.8$  per cent) is significantly higher than the value in the normal trauma series ( $25 \pm 8.3$  per cent), but it is not significantly different from that in the hemorrhage series ( $76 \pm 8.7$  per cent). It should be noted here that on the average all the groups of animals had essentially the same control blood volumes.

*Our data indicate clearly that animals survive muscle trauma with a greater reduction of blood volume if the afferent impulses from the injured area are interrupted by previous deafferentation. The afferent impulses from the injured region must have played an important rôle in bringing about the high mortality of traumatic shock in normal animals.*

In a previous communication (9), significant differences in the clinical manifestation have been reported on animals in which the reduction of the blood volume is effected by simple hemorrhage on one hand and by muscle trauma on the other. The latter group has an early tachycardia often exceeding 200 beats per minute, high mean blood pressure with a rapid terminal decline, high hematocrit values and an early depression of the central nervous system. The clinical picture of deafferented dogs following muscle trauma closely resembles that of simple hemorrhaged animals. As a group, the deafferented dogs had a tachycardia of less than 200 beats per minute or such a heart rate was attained some time after injury. The deafferented dogs were usually not restless, and were not depressed until a short time before death. In these animals the mean blood pressure was usually very low immediately after muscle trauma, but not as low as after hemorrhage. The dog that died with a relatively large residual blood volume (dog D2) not only showed signs of pain, but also had a high blood pressure in the first half



hour following trauma (88 mm. Hg) which was succeeded by a high plateau pressure (100 mm. Hg). In the series of 30 deafferented animals, 14 had mean blood pressures of less than 70 mm. Hg within the first half hour following injury. Six of these survived. It should be recalled that in a large series of over 100 muscle trauma experiments accumulated in this laboratory only 3 dogs survived when the initial mean blood pressure was reduced below 70 mm. Hg by trauma. In the deafferented animals that eventually succumbed, the mean blood pressure was sometimes maintained at a level below 70 mm. Hg for several hours before death occurred. In this respect, the spinal transected animals are impressive in that they maintained a very low level of mean blood pressure for a number of hours without depression of the central nervous system (5). This suggests that the procedure of muscle contusion has two concurrent effects on the mean blood pressure level: *a*, the reduction in circulating volume causes a reduction of the mean blood pressure; *b*, the local somatic afferent impulses produce an elevation of blood pressure. In some animals the latter effect is so dominating that the blood pressure is higher after traumatic injury than in the control. Although the blood pressure of the traumatized normal animal is higher than that shown by the deafferented dog in which an equivalent reduction in blood volume has been produced by muscle trauma, the chances of survival of the former group are no better. Hence, it is the quantity of blood flow to the important tissues rather than the blood pressure alone which is the important factor in determining the fate of shocked animals. This is illustrated further by the observation that the increased blood pressure induced by sciatic nerve stimulation in sublethally hemorrhaged dogs does not help them to eventual recovery. Indeed, such sublethally hemorrhaged animals show a high mortality rate (15). *Our data indicate clearly that afferent impulses excite reflexly the sympathetic nervous system and exert a detrimental effect on the traumatized animals by a further reduction of the tissue blood flow, this being the result of increased vasoconstriction and increased apparent viscosity* (16).

In agreement with the above contention that afferent impulses stimulate the sympathetic nervous system are the changes in the hematocrit values and the serum protein concentrations. It should be recalled that in the simple hemorrhage series (9) there is a decrease in the hematocrit level ( $-4.8$  per cent, from 41.9 to 37.1 per cent) and a dilution of the serum protein concentration ( $-0.9$  gram per cent, from 5.8 to 4.9 gram per cent). In contrast with these findings the traumatized normal dogs show an increase of the hematocrit values ( $+4.1$  per cent, from 42.9 to 47.0 per cent) and no change in serum protein concentrations (5.8 grams per cent). The differences in the average hematocrit changes in the two series can be explained by the leakage of plasma into the region of injury as well as by the increased contraction of the spleen which occur in traumatized animals. The absence of dilution of serum proteins in the traumatized animals indicates not only that the fluid shift is probably small, but also that the plasma lost locally contains a quantity of protein approximately equal to that which enters the circulation from uninjured tissues. The above contention is also supported by the observations on the effects of sciatic nerve stimulation in sub-

ethally hemorrhaged dogs (15). According to these experiments afferent stimulation increases the mean blood pressure and decreases the hemo-dilution which ordinarily occurs after hemorrhage. In the absence of the afferent nerve impulses, one would expect that traumatized animals would show a smaller increase in the hematocrit values and a more appreciable dilution of the serum protein concentrations than would the traumatized animals in which the afferent nerves are intact. The changes which occur in the deafferented animals (an increase in hematocrit values from 40.5 to 42.2 per cent and a decrease of serum protein concentration from 6.1 to 5.6 grams per cent) approach those shown in the hemorrhaged animals, but they are significantly different, because, in the former, leakage of plasma remains an important factor.

The sympathetic nervous system is stimulated by the buffer nerve mechanism in shock produced by hemorrhage or muscle trauma (17, pp. 72-75). It has been shown by Freeman et al. (18) that a totally sympathectomized animal, though able to tolerate a low blood pressure for a long time, is not able to withstand an equivalent volume of blood loss as well as a normal animal. Thus, it is evident that the sympathetic vaso-constrictor effect is a compensatory mechanism, helping to keep the blood flow to the tissues at a relatively high perfusion pressure. The additional vaso-constriction associated with afferent stimulation, which may be initially beneficial in the maintenance of a high blood pressure, may become harmful when the vaso-constrictor mechanism is activated to such a degree that the blood flow to the important tissues is actually impaired (19). This concept is not new (see 20). On the basis of our experiments, the rôle of afferent impulses in experimental traumatic shock is clear. Although they appear to be secondary in importance to the reduction of blood volume, nevertheless they contribute to the death of many of the traumatized animals.

#### SUMMARY AND CONCLUSIONS

In a series of 30 chronic hind leg deafferented dogs, the mortality rate is 50 per cent when the blood volume is reduced to  $64.7 \pm 1.8$  cc. per kgm. of body weight by muscle trauma. This is significantly lower than the corresponding value in the normal trauma series ( $73.4 \pm 3.0$  cc. per kgm., see 9). It is concluded that the deafferented animals are able to survive a greater reduction of blood volume following muscle injury than animals with intact afferent nerves. The afferent impulses from the region of injury must have exerted a deleterious effect on the traumatized animals.

After muscle trauma, deafferented dogs showed a slowly increasing tachycardia, a low initial mean blood pressure immediately after injury succeeded by a low plateau blood pressure, only a slight increase of hematocrit value and a moderate dilution of the serum protein concentrations. They differ from the traumatized normal dogs in that immediately after injury they are less restless and, as shock develops, they show less central nervous depression.

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# BLOOD PRESSURE OF THE RAT DURING ACUTE AND CHRONIC CHOLINE DEFICIENCY

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The possible rôle of faulty nutrition in the pathogenesis of renal and hypertensive disease of animals and man has been suggested repeatedly (1-5). More recently it has been shown that diets deficient in choline produce an acute and striking hemorrhagic renal degeneration with high mortality rate (6, 7, 8). Renal damage may persist for months and may involve glomeruli as well as tubules (7).

Blood pressure not having been studied in such animals it seemed essential to determine whether or not these lesions influence arterial pressure in a fashion similar to that of simple interference with renal blood supply, or whether their effects resemble those of organic lesions such as toxic nephrosis, hydronephrosis, or pyelonephritis in which renal insufficiency frequently progresses to fatal uremia without the appearance of hypertension. It appears that the renal lesions of choline deficiency do not affect blood pressure significantly and to that extent at least they resemble the latter group rather than the former.

**METHODS.** Male weanling rats of the Sherman and Sprague-Dawley strains were used for studying the effect of acute choline deficiency, but both male and female rats were studied in the chronic experiments. Using cages equipped with bottoms made of screening to prevent coprophagy, the experimental animals were placed on the diet described in table 1 either immediately, or after an initial period of 24 hours on Purina laboratory chow. Control animals were fed on the diet listed in table 1 except for the addition of 200 mgm. of choline chloride per 100 grams of diet. All rats had access to diet and water *ad lib.* throughout.

Systolic blood pressures were determined daily in acute experiments, and at least weekly in chronic experiments, using a 10 mm. cuff and the heated plethysmograph previously found to be accurate even when used for weanling rats (10). In the very young, untrained rats during the first week it was necessary to use a hypnotic dose of pentobarbital-sodium (25 mgm. per kilo body weight) intraperitoneally. This reduced spontaneous movement but was not anesthetic, because moderate sensory stimuli still produced movement. A few tests showed that these readings did not differ significantly from those obtained in the same animal without anesthesia.

**RESULTS.** *Acute choline deficiency.* The growth curves of 18 acutely choline deficient and 12 control weanling rats are given in figure 1. Choline deficient rats grew at a slightly slower rate than control animals until the 7th day when they suddenly started to lose weight and fatalities began to occur (inset A, fig. 1.) Fifty per cent of deaths occurred during the 7th to 9th day and the remainder

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by the 12th day. The weight of the animals that died fell progressively during this period. The composite weight curve of the surviving animals was similar to that of those that died except that the loss of weight was not so great. Moreover, on the 12th day the weight of the survivors began to increase, reaching the original level for this group by the 16th day.

In this first group of 18, 6 survived. The mortality of 66 per cent is less than that reported by Engle and Salmon (8). This can probably be accounted for (a) by the smaller amount of peanut flour in the ration (20 per cent as compared to 30 per cent), and (b) possibly by slightly less complete extraction of choline by alcohol due to the larger particulate size of the reground peanut

TABLE 1  
*Diet deficient in choline\**

Peanut Granules† (ground and alcohol extracted).....	20 per cent
Sucrose.....	65 per cent
Casein (Vitamin Test, Smaco).....	6 per cent
Corn Oil (Mazola).....	3 per cent
Cod Liver Oil (Shawmut Brand).....	2 per cent
Phillips and Hart's Salts.....	4 per cent

To 100 grams of the ration were added the following water soluble vitamins‡:

Thiamin.....	0.2 mgm.
Pyridoxin.....	0.2 mgm.
Calcium pantothenate.....	1.5 mgm.
Riboflavin.....	0.4 mgm.
Niacin.....	2.5 mgm.

\* Prepared according to Engle and Salmon (8) as modified by Hegsted et al (9).

† Commercial peanut flour not being available, peanut granules were used. The peanut granules, 1 to 2 mm. size were generously supplied by the Trader's Oil Mill Company, Fort Worth, Texas. This material was ground to pass an 80 mesh screen before extracting the choline.

‡ Water soluble vitamins were supplied by Merck and Company, Inc., Rahway, New Jersey.

meal. Nevertheless all of the survivors had palpably enlarged kidneys during the period of severe illness.

The systolic blood pressures observed in acute choline deficiency are summarized in figure 2<sup>2</sup>. The vertical bars represent the average systolic pressure for the group of animals indicated by the legend in the figure. There was no significant change in blood pressure of the weanling rats during the period of acute choline deficiency; this was true for the dying and also for the surviving animals.

<sup>2</sup> These particular measurements, experimental and control, were made before the optimum temperature for the plethysmograph had been defined (10). Hence the plethysmograph was kept at 40 to 41°C rather than at the optimum of 42 to 44°C, but this does not affect the validity of the comparison here described. These readings should not, however, be taken as accurate absolute values of systolic blood pressure in the weanling rat.

*Chronic choline deficiency.* Weanling male and female rats were placed directly on choline deficient and control (choline supplemented) rations. During the initial period of acute deficiency 66 per cent of the males and 20 per cent of the females died. The survivors, 11 males and 9 females, were continued on the choline deficient diet. The control series consisted of 3 males and 3 female rats. The growth of the deficient animals was distinctly slower than that of the controls and the difference in weights at 5 months was conspicuous (fig. 3).

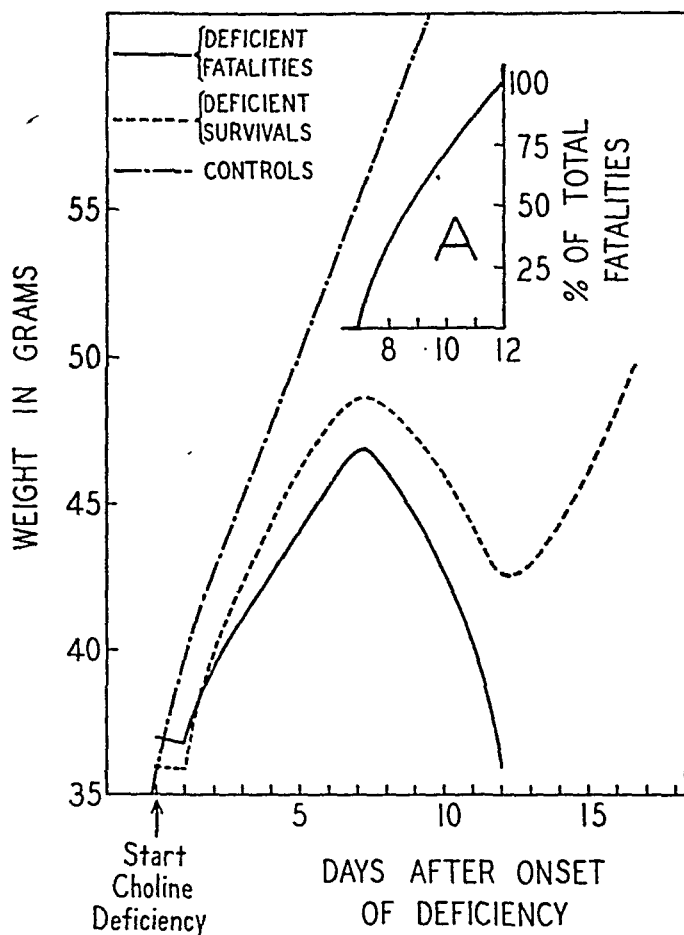


Fig. 1. Growth curves of weanling male rats on choline deficient, and control (supplemented) rations. The continuous line represents average weight of the choline deficient animals that died, the dotted line the choline deficient animals that survived, and the broken line the controls. Inset A is the fatality curve of the animals represented by the continuous line of the growth curves.

As in normal animals the rate of growth and the final weight of the deficient male rats was greater than that of the female rats.

After 3 weeks on the deficient diet a patchy alopecia developed over the head, neck and trunk of first the deficient females, then the deficient males, and finally the control females and males. The lesion was non-inflammatory; the skin at the base of the denuded area was clean and pink. The grade of alopecia varied somewhat with the completeness of extraction of the peanut flour and was sim-

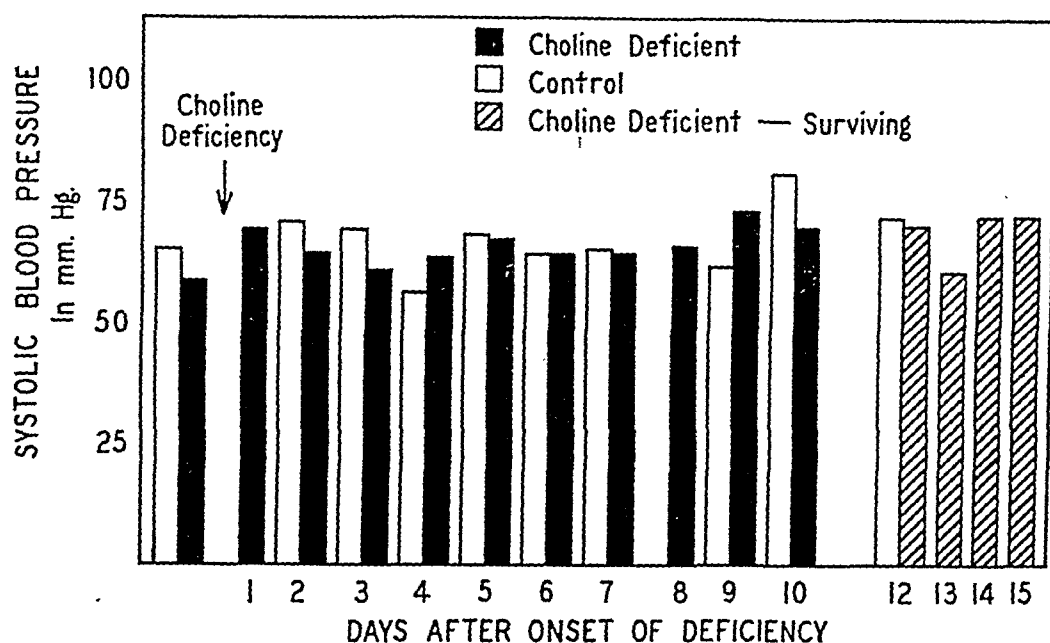


Fig. 2. Systolic blood pressure in acute choline deficiency. Deficient diet started at time indicated by the arrow. These are the average values for the animals represented in figure 1.

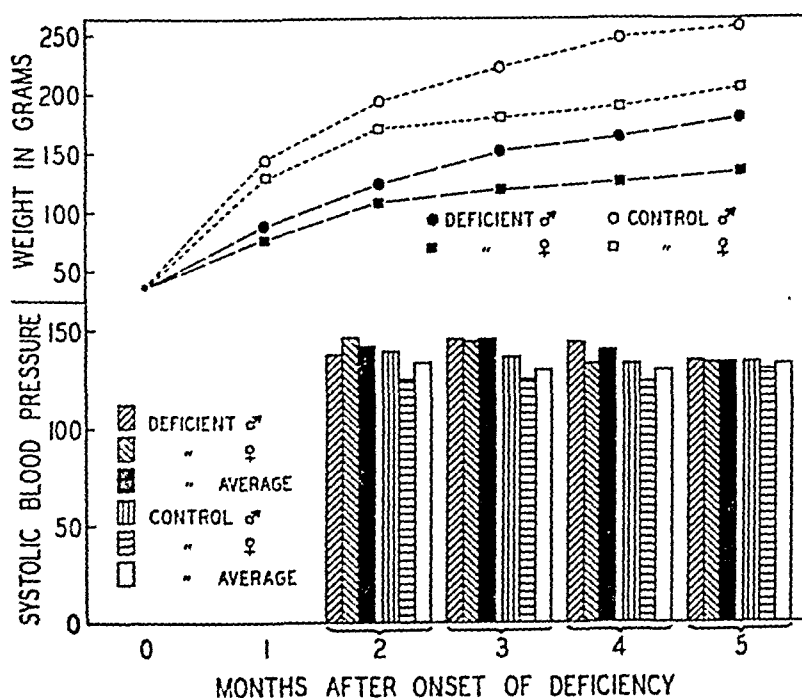


Fig. 3. Upper half. Growth curves for male and female rats during chronic choline deficiency from weaning, and for male and female rats on control diet. Lower half. The average systolic blood pressures of these animals over a period of 5 months.

iliar in appearance to the lesion described with inositol deficiency in the rat (11). Inositol added to the deficient diet in the amount of 0.3 per cent produced marked regression of the alopecia within three days in the one animal thus tested.

Systolic blood pressures were first determined in the entire series when the deficient animals weighed about 100 grams (fig. 3). The average systolic blood pressure of the choline deficient animals was initially 10 to 14 mm. Hg higher than that of the control animals but this difference proved transitory. By the 5th month systolic pressures of both groups were the same. This initial slight difference in systolic pressure may have been due to the marked restlessness of the deficient animals, which also gradually disappeared as they increased in weight and size.

At the end of 5 months the female rats, both deficient and control, were placed for several days with normal males. The control females became pregnant, the deficient females did not. Five deficient females were then placed on the control diet which contained supplemental choline. Their weight increased and pregnancy was then successfully induced in 3 of these 5 previously deficient females. These pregnancies proceeded to term without change of blood pressure. It is not possible to explain the failure to induce pregnancy by a specific inadequacy of the diet other than the prolonged lack of choline, because pregnancy was induced easily in the control animals. The poor general nutrition and the smaller size of the deficient females seems a more satisfactory explanation.

DISCUSSION. Patterson and McHenry (12) have suggested that the "anti-hemorrhagic" property of choline and other substances is closely related to its lipotropic action and to its effect on phospholipid metabolism within the kidney. The need for information concerning blood pressure in choline deficiency was suggested (a) by the severity of the renal lesion; (b), by the known effects on blood pressure produced by interference with renal blood flow, and (c), by evidence that the renal damage of choline deficiency may persist for months and involves glomeruli as well as tubules (7).

Although the term "hemorrhagic renal degeneration" was used by Griffith and Wade (6) for the renal lesion of acute choline deficiency, Gyorgy and Goldblatt (13) described it as an intense cortical tubular necrosis and hyperemia, with hemorrhage only in the subcapsular region. The earliest histologic changes during incipient acute choline deficiency have not been described so far. From such studies in progress it seems possible that the cortical tubular necrosis may not be a primary effect of renal metabolic abnormality per se but rather a secondary mechanical effect of severe and widespread vascular derangement and hemorrhage within the kidney (14).

It is a striking feature of hypertension in man that renal pathology of given type and grade, e.g., pyelonephritis, may in one case be associated with hypertension and in another progress to renal insufficiency without hypertension (15). Apparently this discrepancy exists also in choline deficiency of the rat. Despite severe acute renal lesions, followed by chronic changes involving glomeruli and tubules (7), choline deficiency appears to have no effect on blood pressure.

#### CONCLUSIONS

In rats acute choline deficiency to the point of death, and chronic choline deficiency during 5 months, did not affect blood pressure significantly.



The striking renal lesions of acute and chronic choline deficiency do not belong to that group of renal abnormalities which frequently produce hypertension, but resemble those lesions, e.g., the nephroses, which generally have no effect on blood pressure.

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# IS PROTHROMBIN A UNITARY PRINCIPLE OR A COMPLEX?

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The isolation of prothrombin and the demonstration that it is a homogeneous protein was announced in 1945 (19). Moreover, we have made a search for the possible occurrence in plasma of more than one component of prothrombin, but the results are negative. From the standpoint of chemical isolation, therefore, there is no evidence for the existence of two components of prothrombin.

Our prothrombin can be activated by thromboplastin in the presence of the optimum concentration of calcium ions. Every experiment substantiates the evidence that it is a *complete* prothrombin. From the physiological standpoint, therefore, again there is no evidence of the existence of a prothrombin complex, the two parts of which must be present in order to produce an effect.

Because both the chemical isolation approach and the physiological approach have failed to substantiate the theory that prothrombin consists of two separable components, we have sought an explanation of the results reported by others (4, 6, 10, 22) since there can be no question concerning the reproducibility of their laboratory data when experiments are conducted exactly under the conditions prescribed by them. This brief report serves to throw light upon the suggestion that prothrombin consists of components A and B.

**EXPERIMENTAL.** Analytical results were obtained through the application of standard techniques. The two-stage assay method of Warner, Brinkhous and Smith (21) and the one-stage prothrombin activity determination of Quick (9, p. 312) were used. Desiccated rabbit brain (7) served as the source of thromboplastin in the latter determinations.

Fresh ox blood was collected (20), brought to the laboratory immediately and centrifuged. The supernatant plasma was filtered through a Mandler filter and stored in the refrigerator at 3°C in bottles closed with sterile gauze plugs. Bacterial contamination was avoided. A fresh bottle of plasma was used for analysis each day. Table 1 shows the tabulated results of a series of assays. The two-stage assay method gave results from 190 to 185 units of prothrombin per milliliter over the nine day period; simultaneously the one-stage assay gave results from 100 per cent down to 8 per cent apparent prothrombin activity over the same period.

Since these two methods obviously give extremely divergent results experimental evidence had to be obtained substantiating one or the other of these results. Therefore on the ninth day fresh beef blood was collected and centrifuged as described above. The prothrombin was removed from this fresh plasma by adsorption on  $Mg(OH)_2$  cream (20). This treated plasma was assayed for prothrombin by both methods. Experiment 1 in table 2 lists the results of the

assays, 0 unit prothrombin per ml. by the two-stage and >1200 seconds or 0 per cent prothrombin activity by the one-stage assay. Experiment 2 in table 2 gives the assay of the stored plasma on the eighth day of storage. Experiment 3 gives the assay figures for a mixture of 3 parts of fresh plasma treated with  $Mg(OH)_2$  + 1 part of stored plasma. We found 46 units of prothrombin per ml. by the two-stage and 24 per cent by the one-stage assay. The last line in table 2 shows the dilution correction. The stored plasma contained 184 units of prothrombin per ml. by the two-stage and 96 to 100 per cent activity by the one-stage assay. Under these conditions Quick's method gave results in remarkable agreement with the two-stage determination because reactive fibrinogen was supplied by the  $Mg(OH)_2$  treated plasma.

To confirm this conclusion another experiment was performed with the 8 day

TABLE 1  
*Beef plasma prothrombin determinations*

DAY	ASSAY		
	Two-stage	Quick	
		Clotting time	Activity*
	<i>u./ml.</i>	<i>sec.</i>	%
0	190	16	100
1	190	18	67
2	190	19	62
3	188	23	42
4	190	26	33
5	192	33	23
6	190	42	13
7	188	52	10
8	185	63	8

\* The per cent activity was calculated from the graph on page 37 (9). Since a 16 second clot equals 60 per cent human prothrombin, but is 100 per cent for bovine prothrombin,  $100/60 = 1.67$  the factor used to calculate the values in this table.

e.g., 18 sec. = 40 per cent human prothrombin,  $40 \times 1.67 = 67$  per cent bovine prothrombin activity.

old plasma. Fifty milligrams dry bovine fibrinogen, 98 per cent pure and prothrombin free by test, was dissolved in 10 ml. of the stored plasma and the assays for prothrombin were repeated. Table 3 shows the remarkable reappearance of prothrombin in the one-stage assay and thus convincingly demonstrates that the errors in this technique are due at least in part to the lack of reactive fibrinogen.

DISCUSSION. In 1942 Laverne (2) explained what he believed to be the true failing of the one-stage assay for prothrombin. His conclusion was that fibrinogen, *not prothrombin*, was the labile compound denatured, or partially denatured, during refrigerator storage of human plasma. This change in fibrinogen is responsible for the faulty results obtained when assaying stored plasma by the one-stage technique. Actually Morrison (3) has shown that part

of the fibrinogen precipitates from plasma within a few hours regardless of the anticoagulant used.

However, in 1943 Quick (10) published one-stage assay data on stored plasma prothrombin from which he concluded that "prothrombin is a complex composed of three factors: component A, component B and calcium. By the removal of calcium the prothrombin is dissociated, but on the readdition of ionic calcium a resynthesis of active prothrombin immediately occurs. A diminution of any of these three factors causes a decrease in prothrombin. . . ." The experiments, leading to the conclusion of a two component complex, were done with mixed human and dog plasmas introducing the complications due to species variations. Oneal and Lam (6) published experiments which, they believed, verified the two prothrombin hypothesis. These authors made an additional error. They

TABLE 2

EXPERIMENT	PLASMA	ASSAY		
		Two stage	One stage	
			Clotting time	Interpolated activity
		<i>u./ml.</i>	<i>sec.</i>	%
1	Treated	0	>1200	0
2	Stored 8 day	185	63	8
3	3 pt. treated + 1 pt. stored	46	32	24-25
	Stored—Calculated from 3	184		96-100

TABLE 3

PLASMA	ASSAY		
	Two stage	One stage	
		Clotting time	Interpolated activity
	<i>u./ml.</i>	<i>sec.</i>	%
Stored 8 day.....	185	63	8
Stored + 0.5% fibrinogen.....	185	19	62

interpolated incorrectly the per cent activities of prothrombin from the experimentally obtained clotting times. When the described properties of prothrombin A are compared with those of fibrinogen (1, 3, 5) every chemical property coincides. Similarly, the properties of prothrombin B agree with what is known about prothrombin, the single component molecule.

Quick has published several articles (11-17) based on his original data (10). These publications have been quoted in textbooks and have led Zondek and Finkelstein (22) to postulate two thromboplastins, and Munro et al. (4) to perform hepatectomy on dogs in an attempt to study the two components of prothrombin. These latter workers, in order to explain their data in terms of two prothrombins, concluded that prothrombin A and B are interchangeable. "The reaction between prothrombin A and B appears to be only partially quantitative.

Our observations, . . . , indicate that a deficiency of one component can be partially compensated for by an excess of the other" (4). This illustrates the confusion caused by the two prothrombin hypothesis.

In a communication which has just appeared (18) an experiment is cited similar to Lavergne's (2) and our experiment in table 2, but reversing the proportions of stored and treated plasmas. Moreover, this experiment was done with mixed plasmas, this time human and rabbit, again introducing the complications known to be due to species variations (8, 9, 11, 17, 19). It is very interesting to note that in the experiments where mixed plasmas were used (10, 18), regardless of the proportions or the actual prothrombin concentrations, a 10 second clotting time resulted. From this it is apparent that no conclusions may be drawn as long as heterogeneity of species is dealt with in experiments of this kind.

Another fact that probably has not been sufficiently clarified to date is that since the single component prothrombin, isolated by us, was prepared from oxalated plasma, it would of necessity be only one half of the two component complex. As such it should not be activated with thromboplastin. This, obviously, was not the case. Furthermore, during the past four years, we have isolated prothrombin in as high yields from stored bovine plasma as from fresh plasma.

#### CONCLUSIONS

1. No conclusions concerning prothrombin concentrations may be drawn from work dealing with species heterogeneous mixtures of plasma when the one stage method of analysis is employed.

2. The reactivity of fibrinogen becomes altered in stored plasma.

3. In work with homologous species there is no sound evidence for a second prothrombin component. The evidence indicates that prothrombin is a unitary, stable compound in plasma.

4. In work with homologous species, as demonstrated in the human system by Lavergne and in the bovine system by us, the reactivity of fibrinogen plays an important rôle in the one-stage prothrombin determination.

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# THE DISTRIBUTION AND MOVEMENTS OF CARBON DIOXIDE AND CHLORIDE BETWEEN CELLS AND SERUM OF OXYGENATED HUMAN BLOOD

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Since the pioneer investigations of Hamburger (1) it has been demonstrated repeatedly that the membranes of the red blood cells, of man at least, behave as if they are impermeable to the basic cations, but permit the free passage of water,  $\text{CO}_2$  and chloride (2). The principles which control transfers of chloride and  $\text{CO}_2$  and their distribution between the cells and serum of blood have been examined by several observers (3, 4, 5). Van Slyke and his associates (4, 5) have shown that when the pH of blood is altered by varying its  $\text{CO}_2$  tension, chloride and  $\text{CO}_2$  are exchanged between cells and serum in such a manner that the distribution coefficients of chloride and combined  $\text{CO}_2$  tend to retain a constant relation to one another.

With the exception of Hamburger (1) these investigators have confined their attention almost entirely to the changes induced by varying tensions of oxygen and  $\text{CO}_2$ . The effects of varying concentrations of sodium, potassium, chloride, bicarbonate and other ions on the distributions of  $\text{CO}_2$  and chloride have received little attention. It has seemed worthwhile, therefore, to analyze from this point of view certain data that have been collected for various purposes in this laboratory at intervals in the course of the last 20 years and to compare these with data already in the literature.

**MATERIAL.** The material used for this analysis belongs to several series of experiments of which some have been published. Series 1 consists of 44 samples of blood equilibrated with a tension of 40 mm. of  $\text{CO}_2$  in air at  $38^\circ\text{C}$ ., together with 16 samples which were equilibrated with both 30 and 60 mm. of  $\text{CO}_2$ . These were published by Peters, Bulger and Eisenman (6) in 1923. Series 2 consists of 8 samples of blood equilibrated with 40 mm. of  $\text{CO}_2$  at  $38^\circ\text{C}$ . before and after the addition of known amounts of water or the chloride or carbonate salts of sodium or potassium. These were published by Wakeman, Eisenman and Peters (2) in 1927. To these have been added 9 similar experiments. There are three experiments in which 9 per cent sucrose solution was added to blood and two in which blood after equilibration with 80 mm. of  $\text{CO}_2$  was exposed to incubator temperature for 8 hours.

**METHODS.** The treatment of the materials and the analytical techniques employed have, for the most part, been described in earlier articles. Equilibration of the blood with the desired  $\text{CO}_2$  tension was accomplished by the "second saturation method" of Austin, Cullen, Hastings, McLean, Peters and Van Slyke (7) with the modifications described by Peters, Bulger and Eisenman (6).

Separation and analyses of blood and serum were conducted with strict anaerobic precautions (7). Oxalated blood was used for series 1 and 2, defibrinated blood for the subsequent experiments.

In series 1 and 2 CO<sub>2</sub> was measured by means of the constant pressure Van Slyke pipette, in subsequent experiments by means of the constant volume apparatus. Chlorides of whole blood and serum were measured by the Van Slyke open Carius digestion as modified by Eisenman (8) or by Hald's modification of this procedure (9). The volume of the blood cells was invariably measured by the technique described by Eisenman, Mackenzie and Peters (10). In series 1 and 2 and in some of the subsequent experiments oxygen capacity, determined by the method of Van Slyke and Neill (11), or carbon monoxide capacity was used as a measure of the concentration of hemoglobin. In other experiments the total nitrogen of blood and plasma or serum was measured and hemoglobin was estimated from the protein nitrogen in the blood cells. In series 1 alone was measurement of serum protein omitted. In a few experiments blood and serum were weighed before and after drying to constant weight at 95°. When substances were added to blood the serum or plasma was first separated. The substance to be added was dissolved in or mixed with the serum, which was then returned to the cells. By this procedure hemolysis was avoided.

*Calculations.* The concentrations of all substances in cells were estimated from their concentrations in whole blood and plasma or serum by the formula,

$$\frac{A_b - A_s V_s}{V_c} = A_c$$

in which A = the substance in question, V = the relative volume of the serum or cells, and the subscripts *b*, *s* and *c* represent blood, serum and cells respectively.

The amounts of water in cells and sera, when they were not measured gravimetrically were estimated by the formulae devised by Eisenman, Mackenzie and Peters (10):

$$94.53 - 0.704\text{Hb}_c = W_c$$

$$100.93 - 0.887\text{Protein}_c = W_c$$

$$98.5 - 0.745\text{Protein}_s = W_s$$

in which Hb = hemoglobin and W = per cent of water. In series 1, in which serum protein was not measured, a uniform concentration of 93.3 per cent was assumed for the water of serum.

When materials are added to blood an error in the analysis of one sample is magnified in the processes of calculation by which treated and untreated samples are compared. Certain expedients were, therefore, employed to test the accuracy of the analyses. In most instances both treated and untreated samples of whole blood were analyzed. With the assumption that the amounts of water displaced by the salts were negligible or that added water should be quantitatively recovered, the concentrations of water in the two samples were averaged. In only one experiment did the difference between the two exceed the errors of the methods. Errors of calculation were then tested in the follow-



ing manner. If the amounts of protein in the two phases of the blood remain the same and if water displaced by increments of inorganic elements is negligible, any change in the volume of either phase must represent a loss or accession of water. It follows that

$$\frac{W_s^0 V_s^0 + \Delta V_s}{V_s^1} \text{ should equal } W_s^1, \text{ and}$$

$$\frac{W_c^0 V_c^0 + \Delta V_c}{V_c^1} \text{ should equal } W_c^1$$

when  $\Delta V$  = the change of volume of serum or cells as a result of the treatment and the superscripts 0 and 1 represent untreated and treated bloods respectively. In most instances the values derived by these equations agreed closely with those obtained by analysis. The ultimate validity of all calculations, of course, depends chiefly upon the accuracy of the analysis of serum and the measurements of cell volume. Because of its simpler composition and lower content of protein serum can be measured and analyzed with greater accuracy than whole blood. The measurement of cell volume would seem to be the weakest link in the chain. By taking the average of a large series (4 to 8) of measurements, however, this error was reduced to small proportions, as comparative measures of water indicate. The error of comparative measurements can be evaluated from table 1. Some of the discrepancies in this table are referable to differences between the estimation of water from nitrogen and from direct measurement in the untreated sample of blood.

Combined  $\text{CO}_2$  was calculated from measurements of total  $\text{CO}_2$  at known  $\text{CO}_2$  tension by means of the solubility coefficients of Van Slyke, Sendroy, Hastings and Neill (12). For the calculation of pH the same solubility coefficients were used, together with a  $\text{pK}'_1$  of 6.10 (13). The measurement of  $\text{CO}_2$  is subject to less error than is any other analytical procedure.

The least accurate measurement is that of chloride, the analytical error of which probably slightly exceeds  $\pm 1$  milliequivalent. Since this error may be exaggerated in the calculation of distribution coefficients, it assumes great importance in the comparison of untreated with treated blood. In those experiments in which sodium or potassium chloride were added, however, differences between the amounts of chloride found by analysis in treated and untreated blood can be compared with the differences calculated from the original analyses and the amounts of salt added, which can be measured with great accuracy. This serves as a check on comparative values. The distribution coefficients of chloride in untreated bloods remain the most unreliable features of these experiments. The degree of error in the chloride estimations can be estimated from table 2 in which analytical and calculated values for Cl in treated samples are compared. In the calculation of distribution coefficients in experiments with added chloride calculated values were used whenever there was a great discrepancy. In the bicarbonate experiments analytical values were used; but values for treated and untreated whole blood were averaged.

Concentrations of bicarbonate and chloride are expressed in relation to the water of cells and plasma (or serum). All CO<sub>2</sub> that is not in simple solution is accredited to bicarbonate which should, therefore, properly be termed combined CO<sub>2</sub>. Distribution coefficients  $D_{\text{HCO}_3}$  and  $D_{\text{Cl}}$  are recorded as concentration in serum water : concentration cell water, the reciprocal of the conventional mode of representation, because this form usually yields coefficients between

TABLE 1

*The estimation of cell and serum water in the treated blood samples of series 3*

EXPERIMENT	PER CENT OF WATER IN SERUM FROM				PER CENT OF WATER IN CELLS FROM			
	Analysis for		Calculation from		Analysis for		Calculation from	
	N	Water	N	Water	N	Water	N	Water
1. NaCl added.....	93.7		93.9		64.0		64.1	
2. KCl added.....	94.2		94.4		65.2		65.3	
3. Na <sub>2</sub> CO <sub>3</sub> added.....	93.9		94.0		67.8		68.2	
4. NaCl added.....	93.5		93.6		58.1		68.4	
5. NaCl added.....	94.2		94.4		62.4		62.6	
6. NaCl added.....	94.3	94.0	94.6	94.2	61.3	61.8	61.6	62.0
7. Na <sub>2</sub> CO <sub>3</sub> added.....	94.3	93.3	94.2	93.8	65.3	66.3	64.2	65.1
8. NaCl added.....	92.6	92.8	94.0	93.5	61.8	62.6	61.3	61.4

TABLE 2

*Calculated and analytical concentration of chloride in treated blood samples of series 2*

EXPERIMENT	M.EQ. OF Cl IN				
	Whole blood		Serum	Cells	
	Found	Calculated	Found	Found	Calculated
1. NaCl added.....	*	151.8	187.0		71.8
2. KCl added.....	153.6	154.6	194.8	79.2	82.0
3. Na <sub>2</sub> CO <sub>3</sub> added.....	84.0	83.6†			
4. NaCl added.....	159.5	156.0	178.8	92.8	76.6
5. NaCl added.....	154.4	155.4	192.4	81.4	78.8
6. NaCl added.....	159.2	155.7	191.6	89.6	78.6
7. Na <sub>2</sub> CO <sub>3</sub> added.....	87.0	87.1†			
8. NaCl added.....	155.9	156.4	196.4	80.5	82.0

\* Analysis faulty

† Untreated blood analysis

1.0 and 2.0 which are directly, not inversely, related to pH and to the concentrations of bicarbonate and chloride.

RESULTS. Carbon dioxide was measured in 60 samples of untreated blood and plasma at 40 mm. CO<sub>2</sub> tension and 38°C. The combined CO<sub>2</sub> in the serum of these samples ranged from 79.1 to 28.1 vol. per cent, oxygen capacity from 22.5 to 6.4 vol. per cent, and cell volume from 47.2 to 14.7 per cent. The dots

in figure 1a represent the distribution coefficients of  $\text{CO}_2$  from these measurements plotted against pH. The best line describing the distribution of these points is defined by the equation,

$$(1) \quad 0.569D_{\text{HCO}_2} + 6.68 = \text{pH} \pm \text{s.d.} = 0.060$$

There is no indication in the distribution of the points that the correlation is other than linear. Oxygen capacity and cell volume appear not to influence distribution coefficients.

On the same figure the 30 and 60 mm.  $\text{CO}_2$  tension points are plotted as crosses and open circles respectively. The solid line traversing the figure which represents the best straight line describing the distribution of all 30, 40 and 60 mm. points is defined by the equation,

$$(2) \quad 0.608D_{\text{HCO}_2} + 6.62 = \text{pH} \pm \text{s.d.} = 0.082$$

This differs little from the line describing the 40 mm. points. Nevertheless, with two exceptions the 30 mm. points lie to the right, the 60 mm. points to the left, of the line. Furthermore, in the 2 exceptional experiments the 60 mm. points lie far to the left of their 30 mm. partners and distinctly outside the area of scattering of the 40 mm. points. In every experiment the lines connecting the 30 and 60 mm. points form acute angles with the mean 40 mm. line. The mean slope of these lines (with the exception of one extremely divergent experiment) is 2.494, sharply at variance with the 0.608 of equation 2.

The effects of additions of sodium and potassium bicarbonate are represented in the figure by squares. Since the effects of potassium and sodium proved to be identical (2, 14), the nature of the cation has been neglected. In the three experiments of series 2 only moderate amounts of salt were added; in two later experiments the supplements were large. The tendency for the points representing moderate increments to lie to the right of the mean line is of doubtful significance. Certainly the two points representing large increments straddle the line so closely as to suggest that this line describes over a large range the effect of bicarbonate upon the distribution of combined  $\text{CO}_2$ .

The two experiments marked P in which autoglycolysis was allowed to proceed for 8 hours at 80 mm.  $\text{CO}_2$  tension yielded the most acid bloods and two of the lowest distribution coefficients. Both points lie to the left of the mean line, one far beyond the limits of chance variation.

Water had a somewhat variable action, but the points do not deviate significantly from the limits of deviation of the 64 untreated 40 mm. bloods. The anomalous result in one instance is probably only a mark of the special technical difficulties presented by the water experiments. The sucrose experiments will be discussed later.

The triangles represent the 9 bloods to which chloride was added. To 3 enough salt was added to increase the concentration of chloride by about 40 per cent; in the other 6 it was increased almost 100 per cent. The pH rose 0.02 in 2 experiments, 0.01 in 6; in one it fell 0.01. In all but 2 of the experiments  $\text{CO}_2$  of whole blood rose, the average increment being about 1 vol. per cent,

altogether composed of combined CO<sub>2</sub>. The distribution coefficient,  $D_{\text{HCO}_3}$ , rose 5 times by 0.8 to 1.8, averaging 1.4; it remained unchanged twice, and fell twice by 0.5 and 0.2 respectively. The total average change was +0.63. A change of 0.01 in pH along the mean line should be attended by a change in  $D_{\text{HCO}_3}$  of only about 0.015. The magnitude of the observed changes is too small

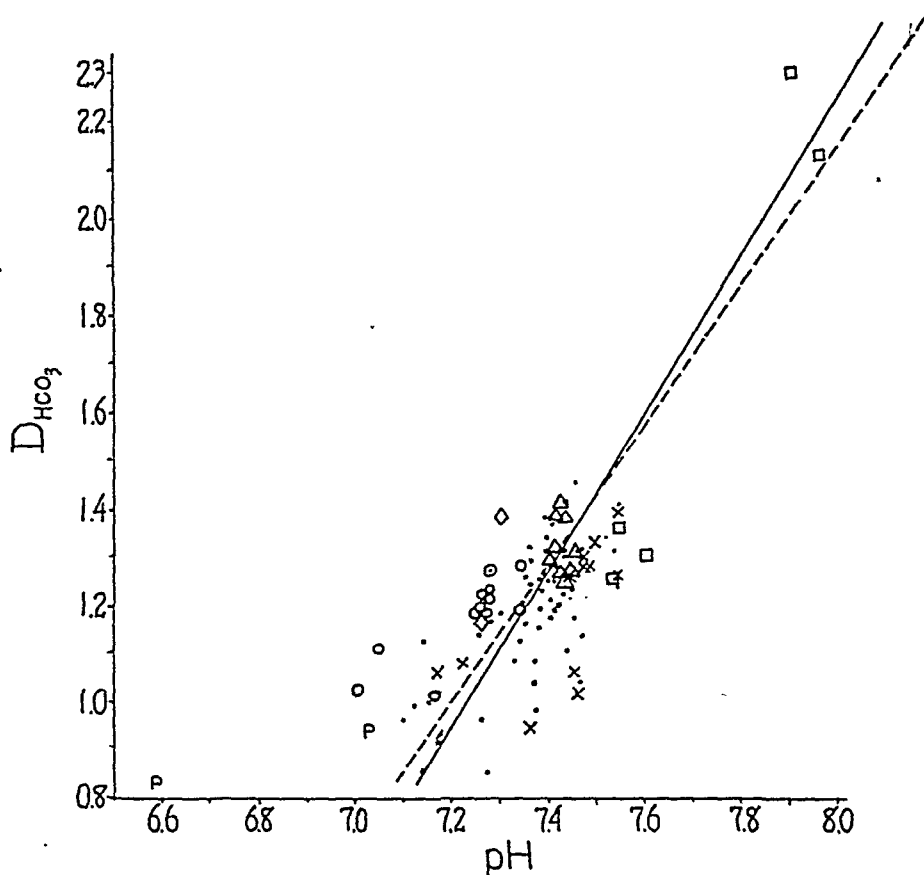


Fig. 1a. The relation of pH to distribution coefficients of combined CO<sub>2</sub> from the authors' data.

Solid circles = blood equilibrated at 40 mm. CO<sub>2</sub> at 38°C.

Crosses = blood equilibrated at 30 mm. CO<sub>2</sub> at 38°C.

Open circles = blood equilibrated at 60 mm. CO<sub>2</sub> at 38°C.

Triangles = blood at 40 mm. CO<sub>2</sub> after addition of sodium chloride.

Squares = blood at 40 mm. CO<sub>2</sub> after addition of water.

Diamonds = blood at 40 mm. CO<sub>2</sub> after addition of water.

P = blood incubated 8 hours at 40 mm. CO<sub>2</sub>.

The mean (solid) line is defined by the equation,  $0.608 D_{\text{HCO}_3} + 6.62 = \text{pH}$ . The broken line, representing the mean distribution of data of Hastings, Sendroy, McIntosh and Van Slyke (5), is defined by the equation,  $0.70 D_{\text{HCO}_3} + 6.50 = \text{pH}$ .

to warrant accurate deductions from so few experiments. The changes appear to exceed the errors of the method. They are far larger than the differences encountered when equal amounts of potassium and sodium chloride were added to separate samples of the same blood. In 2 such experiments whole blood CO<sub>2</sub> differed by 0.6 and 0.2 vol. per cent, and in 4 experiments serum CO<sub>2</sub> dif-

ferred by 0.0, 0.1, 0.4 and 0.1 vol. per cent. The data strongly suggest, therefore, that the addition to blood of neutral sodium or potassium chloride causes pH, combined  $\text{CO}_2$  and  $D_{\text{HCO}_3}$  to rise perceptibly, and the rise of  $D_{\text{HCO}_3}$  is greater than that which would be caused if the pH was increased to the same degree by the addition of bicarbonate.

The distribution coefficients of Cl, in 9 analyses of untreated blood at 40 mm.  $\text{CO}_2$  tension, in which pH ranged from 7.38 to 7.42, varied from 1.34 to 1.67. In the same experiments  $D_{\text{HCO}_3}$  varied only from 1.23 to 1.37. Within this range there was no demonstrable correlation between  $D_{\text{Cl}}$  and either pH or  $D_{\text{HCO}_3}$ . At the mean pH of 7.413, the mean  $D_{\text{Cl}}$  was 1.499, the mean  $D_{\text{HCO}_3}$  1.284, and the mean ratio,  $D_{\text{HCO}_3} : D_{\text{Cl}}$  was 0.856. That pH has a definite influence upon  $D_{\text{Cl}}$  is evident from the fact that the latter rose distinctly after

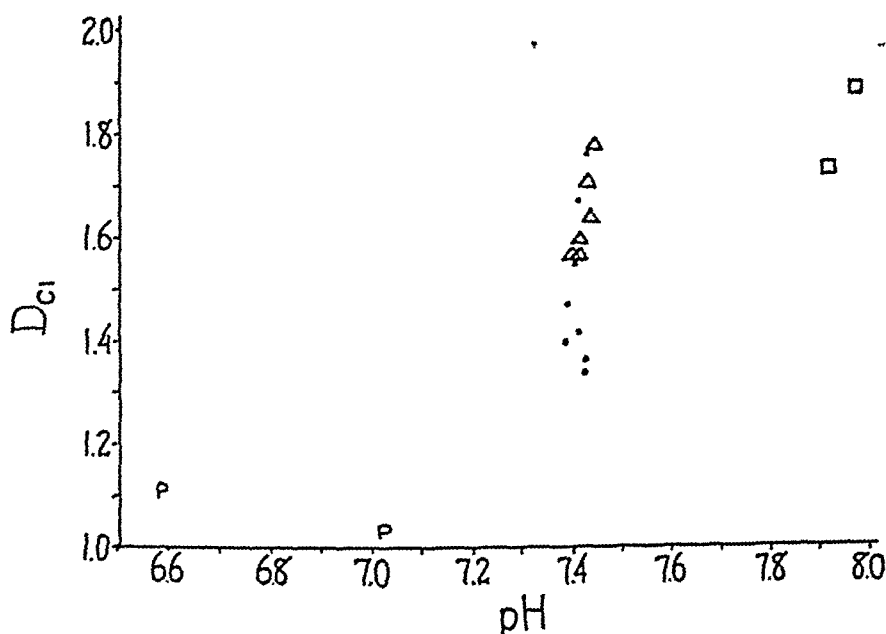


Fig. 1b. The relation of pH to distribution coefficients of chloride from the authors' data. (See fig. 1a for symbols.)

additions of bicarbonate and fell in the autoglycolysis experiments.  $D_{\text{Cl}}$  was, however, affected by these procedures far less than was  $D_{\text{HCO}_3}$ . In the two bicarbonate experiments when pH rose from 7.41 and 7.42 to 7.90 and 7.96,  $D_{\text{HCO}_3}$  rose from 1.37 and 1.25 to 2.30 and 2.13, while  $D_{\text{Cl}}$  went only from 1.41 and 1.34 to 1.74 and 1.83. In the two autoglycolysis experiments pH fell to 7.03 and 6.59,  $D_{\text{HCO}_3}$  to 0.94 and 0.83,  $D_{\text{Cl}}$  to 1.04 and 1.11.

On the other hand,  $D_{\text{Cl}}$  is more affected than  $D_{\text{HCO}_3}$  by addition of chloride. The former rose consistently after this procedure, the increments varying from 0.04 to 0.27, averaging 0.13, while the rise of pH averaged only 0.01.

The sucrose experiments require separate consideration. Sucrose is a neutral compound which does not penetrate the cells (10). It was added in approximately isotonic solution. Its effect, therefore, was to dilute the serum. In a

subsequent paper (14) it will be shown that no base was transferred between the two media of the blood, although enough sucrose was added to increase the volume of the whole blood almost 50 per cent and to approximately double the volume of the serum. Under these circumstances  $D_{Cl}$  fell sharply, in two experiments from 1.49 and 1.48 to 0.73 and 0.85 respectively. These falls, however, do not denote any redistribution of chloride. The amounts of chloride actually found in the serum at the end of the experiment were compared with the amounts in the serum of the untreated blood. The differences, estimated as  $Cl_s^0 V_s^0 - Cl_s^1 V_s^1$  were  $-1.6$ ,  $+0.1$  and  $+0.6$  mM., well within the limits of error of the analytical methods and calculations. In this set of experiments CO<sub>2</sub> of whole blood was not measured so  $D_{HCO_3}$  could not be estimated. It may be surmised, however, that combined CO<sub>2</sub> behaved like Cl, since there was no appreciable change of the quantity of CO<sub>2</sub> in the serum. By the method of comparison used for Cl above,  $CO_{2s}^0 V_s^0 - CO_{2s}^1 V_s^1$  in the 3 experiments was  $-0.38$ ,  $-1.0$  and  $+0.4$  vol. per cent. So long as base and protein are immobilized, therefore, the addition of a nonelectrolyte to which the membrane of the cell is impermeable, in a concentration that causes little or no transfer of water between cells and serum, does not induce any exchange of chloride or CO<sub>2</sub>. It merely dilutes these, together with the other electrolytic components of the serum. The addition of water, which alters osmotic relations in both media, does lead to some exchange of CO<sub>2</sub> and Cl.

The solubility coefficient of CO<sub>2</sub> in the sucrose solution is unknown. If it does not differ greatly from the solubility coefficient of CO<sub>2</sub> in serum, the pH of the serum should have fallen after the addition of sucrose because bicarbonate must have diminished while free CO<sub>2</sub> rose. Since the composition of the cells did not change, on the other hand, the pH within them should have remained constant. Such a discrepancy of hydrogen-ion distribution should be accompanied, according to the Gibbs-Donnan principle, by a proportional change in the distributions of CO<sub>2</sub> and Cl. If it be assumed that the solubility coefficient in the serum diluted with sucrose is the same as that in the untreated serum, the relative changes in pH,  $D_{HCO_3}$  and  $D_{Cl}$  in these experiments can be estimated. For these purposes an approximate value for the initial  $D_{HCO_3}$  may be calculated from the mean line of figure 1a. The results of such calculations are presented in table 3. By comparison with figure 1a it will be seen that the distributions fall far more in proportion to pH than they should if this change were caused by a diminution of bicarbonate. It may be argued that since serum was only diluted by a neutral substance all components were similarly affected. This, the peculiar properties of CO<sub>2</sub> and bicarbonate do not permit. The tension of CO<sub>2</sub> in both treated and untreated specimens was the same. The transfer of bicarbonate that would be required to bring any of these distribution coefficients back to the proper relation with pH far exceeds possible combined errors of analysis or calculation. That pH and distribution coefficients move in the same direction is implicit in the fact that no Cl or CO<sub>2</sub> crosses the cell membrane, and, therefore, may be only a coincidence.

DISCUSSION. In many respects these experiments confirm the conclusions of

Van Slyke and his associates (4, 5). Hastings, Sendroy, McIntosh and Van Slyke (5) measured the distribution of combined  $\text{CO}_2$  and of chloride in 35 samples of blood from normal and diseased subjects. When these data are treated like our own (see fig. 2a) the points assume a linear relation defined by the equation,

$$0.70D_{\text{HCO}_3} + 6.50 = \text{pH} \pm \text{s.d.} = 0.05$$

which agrees well with equation 2 derived from the present data,  $0.608D_{\text{HCO}_3} + 6.62 = \text{pH}$ .

The value of  $D_{\text{Cl}}$  in both sets of data is distinctly and consistently higher than that of  $D_{\text{HCO}_3}$ . The mean ratio,  $D_{\text{HCO}_3} : D_{\text{Cl}}$  in the observations of Hastings et al. (5) is 0.87; in the 8 untreated bloods of our series it is 0.857 at a mean pH of 7.413. The range of pH in these 8 bloods is so small in proportion to the random variations of  $D_{\text{Cl}}$  that no significant relation between them can be discovered. When the treated bloods are added pH appears to affect  $D_{\text{Cl}}$  far less

TABLE 3

*Observed changes of the distribution coefficient of chloride and estimated changes of the distribution of bicarbonate and pH after the addition to blood of isotonic sucrose solution*

	pH	$D_{\text{Cl}}$	$D_{\text{HCO}_3}$
Initial .....	7.44	1.49	1.35
Final .....	7.10	0.73	0.59
Initial .....	7.35	1.48	1.20
Final .....	7.11	0.85	0.67
Initial .....	7.43	1.48	1.33
Final .....	7.14	0.76	0.66

than it does  $D_{\text{HCO}_3}$ . On the other hand, the best straight line describing the data of Hastings et al. (5) is defined by the equation,

$$0.423D_{\text{Cl}} + 6.76 = \text{pH} \pm \text{s.d.} = 0.05$$

(see fig. 2b) which indicates that  $D_{\text{Cl}}$  changes more than  $D_{\text{HCO}_3}$  for a given deviation of pH. Such sharp divergence on a single relation is somewhat perplexing. The range of pH in Hastings' data is relatively small and the arrangement of points somewhat asymmetrical to the line. In view of the greater errors in determination of Cl a larger number of experiments under more varied conditions is required to establish the mathematical nature of this relation precisely.

There can be no doubt that bicarbonate and chloride distribute themselves similarly across the red blood cell membrane as pH changes. It is reasonable to infer that this membrane is permeable to both  $\text{CO}_2$  and to chloride. From studies of horse blood exposed to various tensions of  $\text{CO}_2$  Van Slyke, Hastings, Murray and Sendroy (4) concluded that, as pH varied, the distribution coefficients of combined  $\text{CO}_2$  and Cl remained proportional to one another and to

the distribution coefficient of the hydrogen ion, which they attempted to measure electrometrically. The differences between the three distribution coefficients they attributed to differences in the activities of H<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> ions. Serum probably contains no appreciable quantities of inactive bicarbonate or chloride (small quantities of chloride which may be combined with lipids (15, 16) may be neglected). In this case it can be calculated from the data of Van Slyke et al. (4, 5) that some 30 per cent of the chloride and 50 per cent of the combined CO<sub>2</sub> in the red blood cells is inactive. Since the appearance of Van Slyke's papers the existence in blood cells of CO<sub>2</sub> in other forms than bicar-

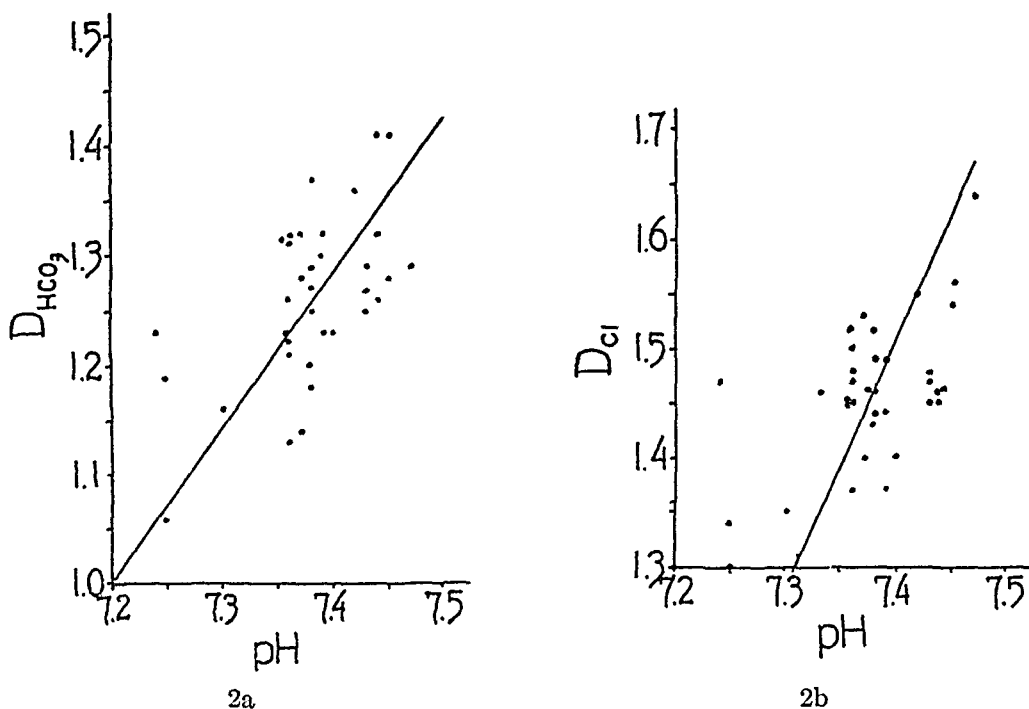


Fig. 2a. The relation of pH to distribution coefficients of combined CO<sub>2</sub> from data of Hastings, Sendroy, McIntosh and Van Slyke (5). The mean line is defined by the equation,  $0.70 D_{\text{HCO}_3} + 6.50 = \text{pH}$ .

Fig. 2b. The relation of pH to distribution coefficients of chloride from data of Hastings, Sendroy, McIntosh and Van Slyke (5). The mean line is defined by the equation,  $0.423 D_{\text{Cl}} + 6.76 = \text{pH}$ .

bonate has been established (17, 18, 19, 20); but the most authentic estimates of the quantities of these forms in cells fall short of the quantities calculated from distribution coefficients. Furthermore, no similar combinations of chlorides in cells have been demonstrated directly. The calculations from distribution coefficients depend upon the distribution of hydrogen ions as a standard of comparison. Actually Van Slyke and his associates measured the pH, not of native cells, but of cells laked with saponin, a treatment that profoundly alters the state of the cellular constituents (21). Their use of the hydrogen ion as a standard of reference must, therefore, be discounted. Additional inferential evidence that there is inactive intracellular Cl can be found in the paradoxical



distributions of chloride and bromide described by Hastings and van Dyke (22, 23).

Whatever the actual state of chloride and combined  $\text{CO}_2$  in the cells may be, the ratios between their distribution coefficients and the relations of each of these to pH can remain constant under all conditions only if the activities of all three components are equally affected by all factors. The dispersion of these distribution coefficients and the variability of their relations to one another in random bloods at the same pH indicate that they are independently influenced by other factors. One of these, the oxygen saturation of hemoglobin, was pointed out by Van Slyke, Hastings, Murray and Sendroy (4). Some others are suggested by the present data.

The mean line in figure 1a, describing the variations of  $D_{\text{HCO}_3}$  with pH, represents the effect on this relation of changes in the concentration in blood of bicarbonate. The tension of  $\text{CO}_2$  was the same, 40 mm., in all experiments except the 12 in which 30 and 60 mm. points roughly balance one another. In the experiments of Hastings et al. (5), in which blood was analyzed as drawn, bicarbonate must also have been the chief variable. The points marking the effects of large additions of bicarbonate straddle the same line. When  $\text{CO}_2$  tension alone was altered in the 30 to 60 mm. experiments a different relation was obtained:  $D_{\text{HCO}_3}$  changed less for a given shift of pH. In this connection it may be remarked that the relation between  $D_{\text{HCO}_3}$  and pH in oxygenated horse blood in table X of the paper by Van Slyke, Hastings, Murray and Sendroy (4) is defined by the equation  $2.075D_{\text{HCO}_3} + 4.69 = \text{pH}$ . This slope is far closer to the average slope, 2.494, of our 30 to 60 mm. experiments than it is to the bicarbonate slope, 0.608. In the subsequent paper by Hastings, Sendroy, McIntosh and Van Slyke (5) on human blood an explanation is offered for the higher values of  $D_{\text{HCO}_3}$  in human blood, but no attention is given to the apparent differences in the slopes relating  $D_{\text{HCO}_3}$  to pH in the two species. The close similarity of the slopes in their horse blood experiments to those of our 30 to 60 mm. experiments suggests that the response of  $D_{\text{HCO}_3}$  to a given change of pH is greater when this is produced by altering bicarbonate than it is when  $\text{CO}_2$  tension is varied. This might be expected since the addition of bicarbonate would presumably alter specifically the active fraction of combined  $\text{CO}_2$ .

The relation of pH to  $D_{\text{Cl}}$  can be discussed with less confidence because of the apparent disagreement between our data and those of Hastings et al. (5). In the former there can be no doubt that  $D_{\text{Cl}}$  rose far less than  $D_{\text{HCO}_3}$  after addition of bicarbonate. In fact, after large additions  $D_{\text{HCO}_3}$  exceeded  $D_{\text{Cl}}$ . The changes in these experiments are quite consistent and the discrepancies between the coefficients exceed all combined errors of analysis and calculation. If they are taken at their face value the fact that the  $D_{\text{HCO}_3}$  and  $D_{\text{Cl}}$  lines cross constitutes circumstantial evidence that there is inactive chloride in the cells.

The addition to blood of chloride salts raises  $D_{\text{Cl}}$  more than it does  $D_{\text{HCO}_3}$ . This treatment caused the concentration of Cl in blood to rise sharply with only minimal changes of bicarbonate and pH. Harkins and Hastings (24) examined the distribution of both chloride and combined  $\text{CO}_2$  in dogs' blood

which had been greatly acidified, both *in vitro*, and *in vivo* by addition of hydrochloric acid. The general distribution of points in all their experiments is defined by the equations,

$$\begin{aligned} 0.979D_{\text{HCO}_3} + 6.085 &= \text{pH} \pm \text{s.d.} = 0.147, \\ 1.101D_{\text{Cl}} + 5.673 &= \text{pH} \pm \text{s.d.} = 0.108. \end{aligned} \quad \text{and}$$

There is nothing to indicate that over a large range these relations are other than linear. The distinction between these and the human curves may be referable to species or to the treatment. In our studies bicarbonate and pH were simultaneously altered by addition of bicarbonate, while Cl remained relatively constant, or else Cl was made to vary while pH and bicarbonate remained relatively constant. In the experiments of Harkins and Hastings, bicarbonate and pH fell while Cl rose. Under these circumstances  $D_{\text{HCO}_3}$  and  $D_{\text{Cl}}$  both changed strikingly with pH,  $D_{\text{Cl}}$  perhaps slightly more than  $D_{\text{HCO}_3}$ .

Ordinarily a small proportion of chloride and a larger proportion of combined CO<sub>2</sub> in the cells appears to be inactive; but the relation of the inactive fraction to the total of each component does not seem to be constant under all conditions. When a salt of either chloride or bicarbonate is added to blood, the distribution coefficient of the added component rises more rapidly than that of the other component because the increment contributes more to the active than to the inactive fraction.

The autoglycolysis experiments introduce further variables: the breakdown of organic phosphate in the cells, the discharge of inorganic phosphate from cells to serum, and the production of lactic acid. The location of these points on figures 1a and 1b is uncertain, since pH was not established. The CO<sub>2</sub> tension from which the H<sup>-</sup> ion concentration was estimated is the tension with which these bloods were equilibrated before they were incubated. During incubation the bloods were preserved anaerobically over mercury. Undoubtedly CO<sub>2</sub> tension rose and pH fell during this procedure as a result of lactic acid production. Oxygen saturation may also have diminished slightly. These factors may be responsible for the shift of the autoglycolysis points to the left of the line relating  $D_{\text{HCO}_3}$  to pH in figure 1a. The possibility can not, however, be excluded that this is the effect of the high CO<sub>2</sub> tension, 80 mm., to which these bloods were exposed. The effects of other anions on the distribution of bicarbonate and chloride deserve exploration.

The sucrose experiments are peculiarly disconcerting. They emphasize the fact, too often overlooked, that the close relation—almost identification—of osmolar concentration with electrolyte concentration in serum is no expression of a great principle, but an accident or coincidence. The dominance of serum electrolytes over the movement of water between cells and serum belongs to the same class of phenomena. If nonelectrolytes incapable of crossing the cell membrane existed in high concentration in the serum, electrolytes would no longer serve as a measure of osmolar concentration. If the concentration of immobilized nonelectrolyte varied, the obligation of water to electrolytes would be proportionately reduced. If blood is regarded as a system in which two

media are separated by a membrane impermeable to cations and a large proportion of anions, but freely permeable to water, it is clear that the addition to blood of an isosmolar solution of a nonpenetrating nonelectrolyte should have no other effect than to dilute in the serum those electrolytic components which are not restrained. On the other hand, the Gibbs-Donnan principle would require that anions and cations to which the membrane is permeable should redistribute themselves according to a definite rule which, in blood, appears to be violated. This does not mean that the Gibbs-Donnan principle is false. Such an interpretation would imply that biological systems can circumvent thermodynamic laws. It means that the full dimensions of the equilibria between blood cells and plasma—and between cells and their fluid environments in general—have not yet been explored and that efforts to reduce them to even approximate mathematical terms of physical chemistry have thus far been successful only when the dimensions and limits of variation have been greatly restricted. The effects of other isotonic solutions on distribution coefficients require examination as do the effects of varying concentrations of anions other than bicarbonate and chloride.

#### CONCLUSIONS

The distributions of chloride and combined  $\text{CO}_2$  between cells and plasma of oxygenated blood have been altered by varying  $\text{CO}_2$  tension, by adding chloride or bicarbonate salts of sodium and potassium, water and isotonic sucrose solutions, and by autoglycolysis.

Although the distribution coefficients vary with pH in the general manner described by Van Slyke and his associates (4, 5), these variations seem to depend also upon the manner in which the pH is altered. Reasons for these different reactions have been discussed.

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## THE CORONARY CIRCULATION IN THE DOG<sup>1</sup>

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In a recent review Gregg (16) has pointed out that although coronary blood flow has been measured by a variety of methods and under many different conditions, practically all of the studies made in the past are open to criticism. More specifically, if the instrument used to measure coronary blood flow was capable of dependable accuracy, the measurements were made under abnormal conditions such as artificial respiration with the chest open, or a heart-lung or coronary perfusion preparation. If the experimental conditions approximated the normal, the measuring device was usually the thermostromuhr, the dependability of which is questionable (15). Gregg concluded (16) that most of the work already done in this field should be repeated with the more accurate instruments now available and under conditions more closely approximating the normal.

The experiments described below were undertaken with this purpose in mind. The instrument used to measure coronary blood flow was the bubble flowmeter previously used in studies of the cerebral circulation (5, 23). This device is reasonably accurate and dependable. It has the great advantage over instruments such as the rotameter, venturimeter, and orifice meter of being practically unaffected by changes in blood viscosity or deposition of flecks of fibrin during the course of an experiment. Another advantage is that the bubble meter does not introduce any appreciable resistance to the flow of blood through the coronary system. This was proved by observations both on mercury manometers connected by T tubes proximally and distally to the flowmeter, and on the volume of blood passing through the meter when it was connected to a femoral vein instead of a coronary artery. Experiments of the former type showed no measurable difference in the two lateral pressures; the latter gave flows exceeding 150 cc. per minute at ordinary arterial pressures—a value almost double the most rapid coronary blood flow encountered in these experiments.

The experiments were necessarily made on anesthetized heparinized dogs but in most cases the conditions were superior to those under which measurements of comparable accuracy have been made in that the chest was closed and the animal was breathing spontaneously.

**METHOD.** Dogs, unselected as to breed and sex and averaging 13 kgm., were initially anesthetized with nembutal (32 mgm. per kilo intravenously) or chloralose (50 mgm. per kilo intravenously) following morphine (2 mgm. per

<sup>1</sup> The expenses for these experiments were defrayed in part by a grant from the Life Insurance Medical Research Fund.

kilo subcutaneously). Anesthesia was kept light, a positive corneal reflex usually being present. Blood pressure was recorded from one femoral artery with a mercury manometer. A tracheal cannula was inserted and under artificial respiration the chest was opened either by the removal of a portion of the left fifth rib or by a direct approach through the 4th interspace. The pericardium was grasped with forceps and opened laterally by cautery to prevent bleeding from the minute pericardial vessels. The cut edges of the pericardium were then sewed to the chest wall which brought the heart into an easily accessible position. If the anterior descending coronary was to be cannulated, a small incision was made through the epicardium directly over this artery, and

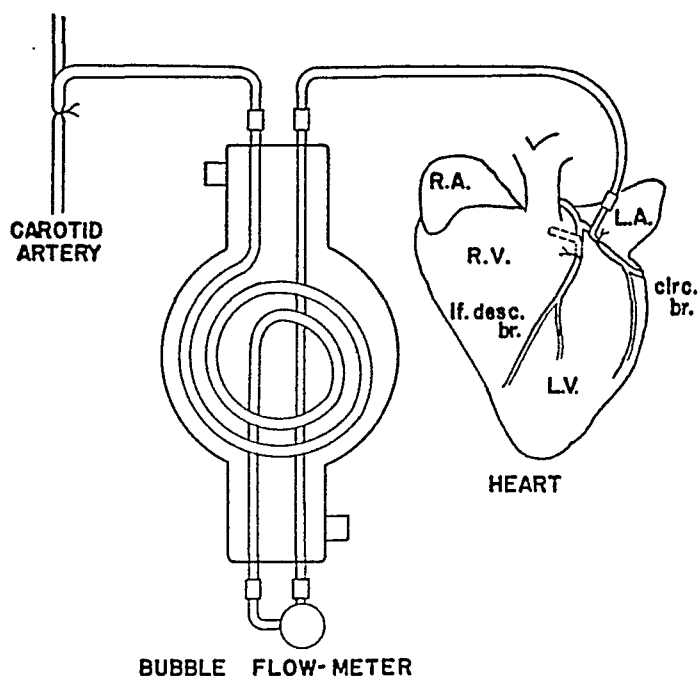


Fig. 1. Schematic representation of the experimental arrangement. (See Dumke and Schmidt (5) for details of the bubble meter.) *Lf. desc. br.*—anterior descending branch of the left coronary. *Circ. br.*—circumflex branch of the left coronary. The dotted cannula indicates position when the anterior descending branch is cannulated.

the artery was freed from the surrounding tissue by blunt dissection. Care was taken to see that the ligatures were passed around nothing but the artery and that as short a section as possible was freed, usually 5–7 mm.

If the circumflex coronary was to be cannulated, the left auricular appendage was grasped with a serrefine and retracted from the field. The artery was freed from the surrounding tissue in the same fashion as above. Cannulation of the circumflex was usually accomplished within 5 mm. of the bifurcation of the left coronary and cannulation of the anterior descending within 1 cm. (fig. 1).

After the ligatures were in place, the animal was given heparin (350 units per kilo) and this dose was repeated every hour. The bubble meter was connected

by glass tubing to a cannula inserted into the right carotid artery. The entire system was filled with blood up to the tip of the glass coronary cannula. The artery was then quickly tied proximally, cut, the cannula inserted, blood allowed to pass through the meter into the coronary by releasing the clamp on the carotid inflow, and the cannula then tied into place. Blood flow was stopped in the artery for periods varying from 50 to 150 seconds with 85 seconds being the average.

After cannulation was completed an interval of fifteen to twenty minutes was allowed to elapse for the flow to stabilize. Following this the flow was recorded, the chest closed, the pneumothorax reduced and the animal allowed to resume spontaneous respirations.

Intratracheal oxygen, 3 liters per minute, was delivered routinely throughout the experiment except where precluded by the procedure. This minimized the possibility of anoxia influencing the coronary flow which might conceivably have occurred without being recognized.

Flow per gram of heart tissue was determined by a terminal injection of Evans Blue dye into the stream flowing through the coronary artery. The animal was sacrificed by an intravenous injection of 25 per cent magnesium sulfate solution and the whole heart as well as the dyed portion of the heart was weighed. The results of these injections are shown in table 1. The weight of the areas supplied agrees with the data of Anrep (2) in which he measured the inflow into each coronary artery from graduated reservoirs. The fact that our percentages for the average weight of heart tissue supplied by the anterior descending and circumflex coronary arteries are somewhat lower than Anrep's figures for the percentage of blood to the heart carried by those arteries is to be expected since Anrep was careful to include the entire artery and all of its branches when measuring the flow. In our particular problem it was not felt necessary to sacrifice the time needed to have the cannula supplying all of the branches near the bifurcation. Consequently some of these small branches might have continued to receive blood directly from the coronary artery and not through the bubble meter.

On a few occasions injection of the heart was made after the animal's death but there was no significant difference in the relative weight of the dyed area measured in this way from those experiments in which the injection was made antemortem.

The nature of the bubble flowmeter makes the intracoronary injection of drugs very simple. The drug to be investigated was injected through a small valve in the tube leading from the meter into the heart. Each injection was standardized to 0.2 cc. total solution and a 2 second period used for administration. The syringes, needles, and solutions were kept in a water bath at 38°C. Dilutions were made with Ringer-Locke solution. Control injections of this solution resulted in no significant alteration in coronary flow.

Successive readings of coronary flow under unchanging conditions have not varied more than 7 per cent; the usual variation was 1-3 per cent. No change

of flow is considered significant unless it is directional and is greater than 7 per cent.

RESULTS. *Volume of "normal" coronary flow.* Under the conditions of these experiments the volume of blood passing through the coronary arteries was approximately 65 cc. per 100 grams of heart per minute. As shown in table 2 the figure is fairly consistent under either closed or open chest conditions.

TABLE 1

	NUMBER OF EXPERIMENTS	AVERAGE WEIGHT (GRAMS)		AVERAGE % A/B	COEFFICIENT OF VARIATION
		A Entire heart	B Injected area		
Anterior descending coronary..	18	113 Range 92-200	30.0 Range 18-60	26.2	$\pm 4.82$
Circumflex coronary.....	17	102.5 Range 79-154	41.5 Range 29-60	40.6	$\pm 3.56$

TABLE 2

*"Normal" coronary blood flow*

	NUMBER OF EXPERIMENTS	FLOW CC/100 GRAMS/MIN.	COEFFICIENT OF VARIATION
A.—Flow measured with blood pressure at the same level as at the beginning of the experiment			
Closed chest.....	11	64.70	$\pm 16.3$
Open chest.....	7	59.00	$\pm 24.4$
B*.—Flow measured with blood pressure below the level at the beginning of the experiment			
Closed chest.....	27	55.60	$\pm 23.7$
Open chest.....	26	51.00	$\pm 31.0$
C*.—B with flow corrected to blood pressure level at the beginning of the experiment			
Closed chest.....	27	64.00	$\pm 22.4$
Open chest.....	26	63.00	$\pm 30.4$

\* A included in B and C.

Although Essex and his co-workers (7) using the thermostromuhr in otherwise intact dogs could find no such consistent relationship between coronary flow and heart weight, Katz et al. (19) using the heart-lung and isolated heart preparations, in spite of considerable variations in coronary flow per gram of heart tissue, concluded that there was a definite correlation between coronary flow and heart weight. Wiggers (26) has given the figure of 50-75 cc. per hundred grams per minute which is in full accord with our "normal" value.



Of the 27 closed chest experiments included in this report, in only eleven did the blood pressure return to its previous levels after the artery was cannulated and the chest closed (table 2 - A). In those experiments in which the pressure remained at a lower level one can accept the coronary flow at that level (table 2 - B) or one can make a correction for the change in blood pressure (table 2 - C). As will be shown in the next section, there is a consistent relationship between blood pressure and coronary flow and the correction is made on this basis. Even if one does not wish to make this correction, the table shows that the flow relationship between A, B and C is very close.

It is interesting to note that there is no significant difference in coronary flows between open or closed chest conditions although the former tend to be more variable. This factor alone would not therefore disqualify otherwise valid measurements of coronary flow.

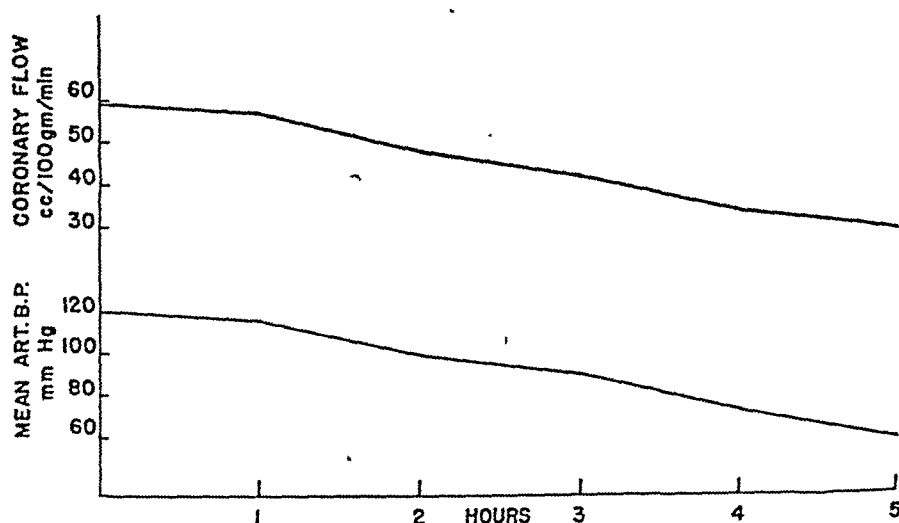


Fig. 2. Relationship of blood pressure and coronary flow. Average 12 experiments. The graph represents data from the first 12 experiments. The subsequent experiments gave comparable results.

*Factors which may influence coronary flow.* 1. *Blood pressure.* The relationship between mean arterial blood pressure and coronary blood flow in the first twelve experiments is shown in figure 2. It is evident that as blood pressure fell there was a corresponding fall in coronary blood flow. Although there is no increase in blood pressure recorded in the figure, such increases have occurred (but are cancelled out in obtaining averages) and when they occur are associated with an increase in coronary flow. Similar responses were encountered in the remainder of the experiments. Such a relationship has been repeatedly encountered (17); our data serve to confirm and emphasize it.

The effects of blood pressure on the coronary flow were evident during individual experiments. With slight spontaneous changes in blood pressure there was an instant reflection in the rate of coronary flow. When Traube-Hering waves in the blood pressure were encountered, there were corresponding vari-

ations in the flow which were consistently in the same direction as the pressure changes.

2. *Heart rate.* No attempt was made to control this factor by electrical stimulation of the heart as this would have made the experiments highly artificial. A valid correlation could not be deduced from a series of experiments but in individual cases we encountered evidence of the influence of heart rate upon coronary flow. One of these is shown in table 3. In this animal there was a spontaneous increase in heart rate which is not uncommon under the type of anesthesia used (morphine-chloralose). Coronary flow increased as the heart rate became faster and the association gains in significance because it is contrary to the concomitant changes in blood pressure. A similar relationship was seen in other experiments and from our present data we regard it as the typical one. However, the effect was never marked and could be readily overcome or accentuated by changes in blood pressure.

This relationship of cardioacceleration and increased coronary flow is not supported by the results of Anrep and Hausler (1) and Gregg (14) who found a

TABLE 3

*Effect of heart rate upon coronary flow*

EXPERIMENT 27—ANESTHESIA MORPHINE = CHLORALOSE								
Pulse.....	88	100	112	116	128	120	132	140
Flow cc/100g/min. . .	40.5	41.0	44.3	43.0	45.0	42.7	47.3	46.8
Mean art. B.P.....	138	135	138	135	134	130	128	127
Time.....	5:13	5:14	5:15	5:17	5:20	5:21	5:24	5:25

slight decrease in flow with acceleration in heart rate. The work of Essex et al. (8) is in agreement with our findings.

3. *Vasomotor control.* Under the conditions of these experiments no significant coronary vasoconstrictor activity could be demonstrated by the stimulation of the vagus or accelerator nerves. Stimulation of the accelerator nerves has been accomplished 27 times in 6 experiments and has invariably led to an increase in coronary flow (table 4). This increase was usually associated with a rise in blood pressure and an increase in heart rate, but in several experiments when there were no increases in blood pressure and only minimal changes in heart rate, the acceleration of flow was still noted.

The effect of the vagus has been tested 50 times in 12 experiments. Stimulation of the peripheral end of the cut cervical vagus did not give consistent results, probably for the reasons advanced by Greene (12). If the current (Harvard Inductorium) was strong enough to cause a fall in blood pressure, there invariably was a slowing of coronary flow. When pressure changes were minimal, yet strong vagal activity was evident, as shown by slowing of the heart rate, there was no significant alteration of coronary flow. The few times a slight decrease in flow has been observed could be explained on the basis of changes in heart rate. Following atropine, vagal stimulation did not result

in a consistent diminution of coronary flow such as reported by Greene (12). However the discrepancy may be explained by the difference in methods. We have first determined the threshold stimulus needed to produce asystole with the cut cervical vagus. After atropinization the nerve was again stimulated at the same threshold stimulus. Usually this threshold was 12 cm. Greene consistently used a stimulus between 4-8 cm. which we did not use because it was associated with respiratory changes, muscle twitchings, and changes in tone of the abdominal wall, all apparently due to spread of electrical activity. We could not be certain that a change in coronary flow under these circumstances was due to any one factor.

In 8 experiments in which atropine was administered intravenously (2 mgm. dose), alterations in coronary flow were in either direction, increasing or decreasing with similar changes in blood pressure. In none of these 8 experiments did atropine give any clear cut evidence of increasing coronary flow such

TABLE 4  
*Effect of autonomic nerve stimulation upon coronary flow\**

NO. EXPTS.	NO. TRIALS	MEAN ART B.P.			PULSE			FLOW CC/ 100G/MIN.		
		Before	After	% Change	Before	After	% Change	Before	After	% Change
Accelerator stimulation										
6	27	106	115	+8	142	171	+20	53.6	65.1	+21
Vagus stimulation										
14	20†	96	93	-3	146	124	-15	41.8	38.5	-8
Vagus stimulation after atropine										
3	5	87	85	-2	185	185		44.3	43.7	-1.5

\* Mean response of number of trials indicated.

† Includes only those trials in which a definite vagal response could be obtained.

as reported by other workers (8). Since the heart rate in most of these experiments was about 140-160 before atropinization, there was very little further change in the rate after atropine, so the factor of change in coronary flow due to increase in heart rate was eliminated. This may explain our results.

Ligation of one coronary artery has been reported to lead to reflex vasoconstriction in the other coronary arteries (21). We have not been able to find any evidence of such a reflex in 5 experiments in which the coronary flow was measured in the circumflex branch when the anterior descending coronary was occluded. In all but 1 of 8 trials, the flow in the circumflex increased within one minute after occlusion, even though in 5 trials the blood pressure began to fall immediately. In all trials as the blood pressure continued to fall, the flow began to decrease. Similar results were obtained in 3 trials after the animal was atropinized. The eventual fall in flow was probably due simply to the concomitant decrease in blood pressure.

Gilbert et al. (9) have reported data showing an increase in abdominal visceral tension to be associated with a decrease in coronary flow. This has been ascribed to coronary vasoconstriction. We have increased the intra-biliary tension 13 times in 4 experiments.<sup>2</sup> This was accomplished by inserting a small balloon into the fundus of the gall bladder before the coronary cannula was inserted. When the tension was to be increased, water was injected into the balloon. Under the conditions of these experiments the immediate response of the coronary flow was variable and was always in the same direction as the blood pressure change. In no instance did the blood pressure and the coronary flow go in opposite directions. The most commonly encountered response was a fall in blood pressure associated with a considerable augmentation of the heart rate. Following atropine, the changes of flow, blood pressure, and heart rate were not so marked.

We realize that we are dealing with a cut artery and the effect of cutting and ligating must remain an unknown factor for the present. We were very careful, however, to separate from the artery any strands of tissue that might be nerves and to exclude them from the ligatures. According to Cannon (3), the vasomotor supply of a tissue comes from too many sources to be interrupted, even by an intentionally complete periarterial sympathectomy, but the applicability of these remarks to the coronary distribution is problematical.

Subject to these limitations we feel that our work proves that, if vasoconstrictor nerves to the coronary artery exist, they exert no important control over the amount of blood passing through the arteries. We are in complete agreement with Greene (13) in his statements stressing the lack of importance of the vagus as a coronary constrictor, and our findings with the accelerator indicate that it definitely does not carry any constrictor fibres to the coronaries.

*The effect of acetylcholine and epinephrine.* Since the generally accepted theory of autonomic nerve transmission is based on the liberation of acetylcholine and an epinephrine-like substance, our findings with these agents are of interest in connection with the coronary innervation. A number of other drugs were also tested but the results are reported elsewhere (6).

*Acetylcholine.* This drug was injected into the coronary artery 22 times in 4 experiments. Small amounts ranging from 0.02 to 2.0 micrograms produced an immediate and marked acceleration of flow which returned to its previous levels within 1 to 3 minutes (fig. 3). If the dose was properly chosen this response was obtained without significant changes in blood pressure or heart rate. Larger doses usually elicited a considerable immediate increase in flow followed by the usual transitory asystole accompanied by a slowing of the coronary flow. Not uncommonly 2.0 to 20.0 micrograms of acetylcholine increased the flow followed secondarily by an increase in heart rate. After atropine, acetylcholine, in the dosage used, had no effects of any kind, since the amounts were too small to overcome the atropine or to elicit nicotinic actions.

<sup>2</sup>Morphine-chloralose anesthesia was used. The effect the morphine had upon sensation under these circumstances is questionable.

When acetylcholine was injected into the systemic venous circulation, coronary flow was always decreased. The concomitant hypotension evidently was the dominant factor.

*Epinephrine.* Epinephrine was injected in varying dosage 36 times in 8 experiments. The response to the intra-arterial injection was very similar to that of acetylcholine except that the increase in flow was usually not so great and the duration of action was somewhat longer (fig. 3). The response was also more variable from animal to animal. Thus, in one dog a small dose (0.02 to 2.0 micrograms) led to a considerable increase in flow without a significant change in heart rate or blood pressure, while in another animal the same amount caused a marked acceleration of the heart rate and a precipitous drop in blood pressure.

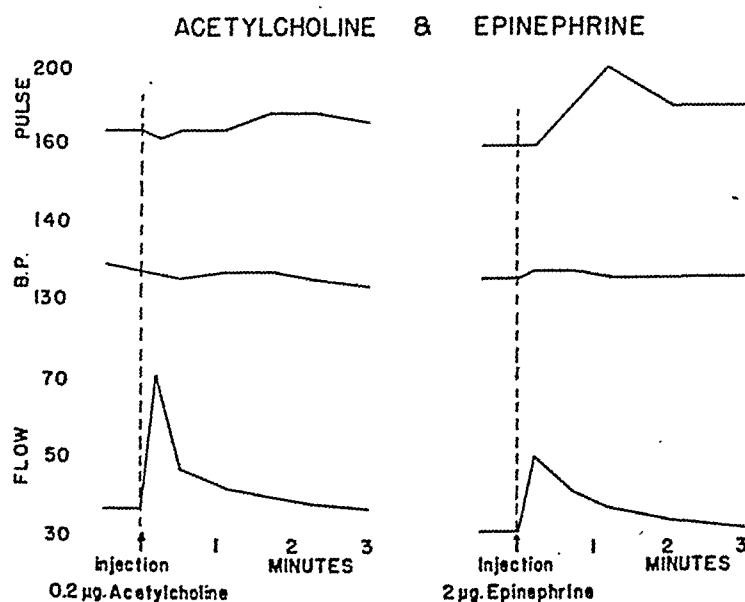


Fig. 3. Pulse = beats/min. B.P. = mean arterial blood pressure. Flow = coronary flow in cc./100 G./min. Injections were intra-arterial.

Green (10) injecting epinephrine intra-arterially was unable to obtain an effect if less than 1 microgram was used. We have used 0.2 microgram 9 times in 9 dogs with an average increase of 35 per cent in flow and have used 0.02 microgram 7 times in 5 dogs with an average increase of 8 per cent. The fact that in most of the small dose ranges a significant increase in coronary flow is seen without changes in heart rate or blood pressure leads us to conclude that epinephrine does have a significant dilator effect per se upon the coronary vessels. However, in the higher dose ranges we are entirely in agreement with Green (10) in concluding that much of the increase in flow is due to increased cardiac metabolism. In this dose range (50 to 500 micrograms) intravenous injections have led to as much as an 880 per cent increase in coronary flow, but this was associated with a 320 per cent increase in blood pressure.

It has been claimed (10) that much of the dilator action of epinephrine may be due to the preservative contained in the solution. In these experiments we

used ampules of epinephrine hydrochloride for parenteral administration with sodium bisulfite as the preservative. While bisulfite itself is a dilator, we found that the amounts required are far greater than those present in the dilutions of epinephrine which produced typical responses. We also used solutions of synthetic epinephrine without any preservative and obtained the usual response.

4. *Cardiac output.* The relationship of coronary flow to cardiac output was investigated by 38 determinations of cardiac output in 17 experiments. The direct Fick method was used, substantially as employed by Shore et al. (24). The results are summarized in figure 4. They indicate that the fraction of the cardiac output passing through the coronary arteries varies inversely with the cardiac output. In the animals under our standard conditions, the heart received 4-5 per cent of the total cardiac output. In all instances as the output decreased,

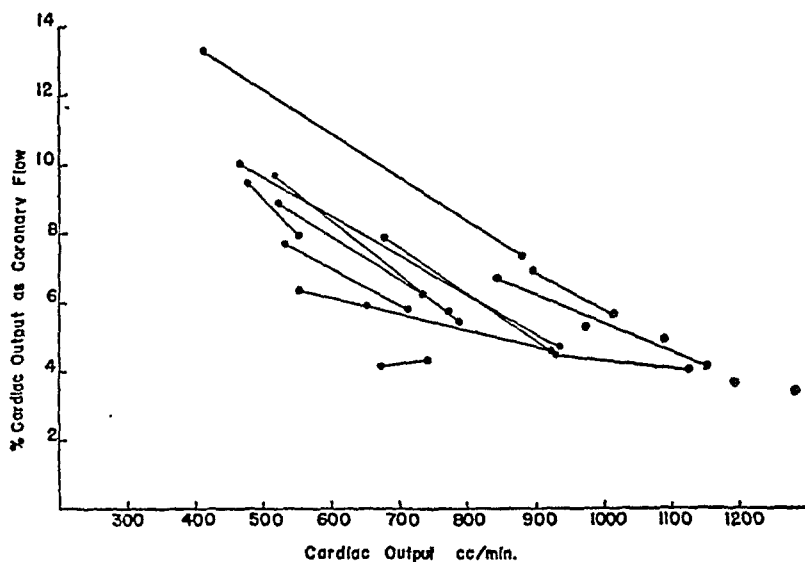


Fig. 4. Relationship of coronary flow to cardiac output. Dots connected by lines indicate observations in the same experiment. Dots unconnected indicate experiments in which only one determination of cardiac output was made.

the percentage of blood being delivered to the coronary circulation increased, so that with outputs of 500 cc. or less per minute more than 9 per cent of the total flowed through the arteries of the heart. The actual flow per 100 grams of heart usually decreases as the output falls.

Katz, Wise and Jochim (19) concluded from experiments on the heart-lung and isolated heart preparations of dogs that cardiac output is of prime importance in determining coronary blood flow. Our data do not permit of such conclusions. Cardiac output, peripheral resistance, and blood pressure are so closely associated and are so interrelated that this type of experiment does not lend itself to the study of the individual importance of these factors.

5. *Changes in blood gas tensions or in acidity.* The findings thus far reported confirm the already general belief that the coronary circulation resembles the

cerebral in susceptibility to changes in general blood pressure and immunity to marked direct effects from vasomotor nerves. Considerable interest therefore attaches to the possibility that the similarity might extend to a similar responsiveness to chemical products of metabolism which have been clearly established as the dominant factor in the intrinsic control of the cerebral circulation in animals (5, 23) and man (20). The data now to be presented justify an affirmative answer to the general question. They also indicate some points of difference that appear to be significant.

A. *The effect of ischemia.* When the coronary artery was occluded for a brief period the flows recorded immediately after release were invariably greater than they were before or afterward at the same pressure (fig. 5). This indicates that the coronary vessels, like the cerebral, are dilated by the accumulated

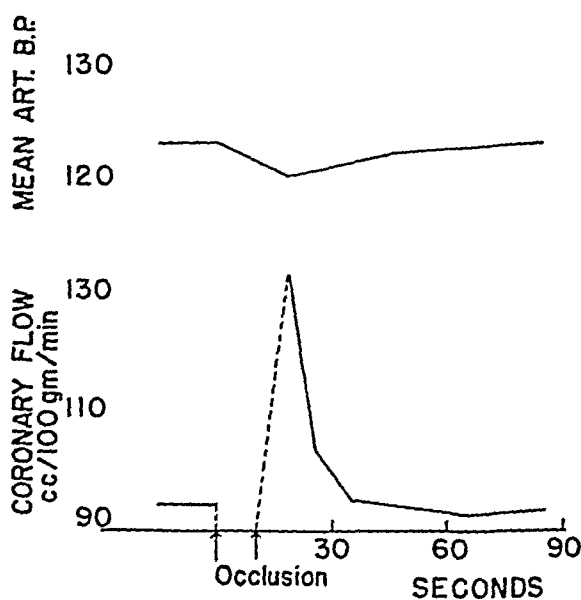


Fig. 5. Response of the coronary artery to brief occlusion. Arrows indicate beginning and end of 10 second occlusion of coronary artery. Mean arterial blood pressure is in mm. Hg.

products of local metabolism. Added evidence in the same direction was obtained from the experiments in which cardiac output was measured. As noted above, the fraction of cardiac output diverted to the coronaries increased as the cardiac output decreased. Further examples of this course of events during shock are given in table 5.

B. *The effect of arterial oxygen content.* Oxygen in 100 per cent concentration has consistently proved to decrease coronary flow. As noted in table 6, in ten experiments in which 100 per cent oxygen was given (from a spirometer or breathing bag) without fall in blood pressure, there was an average reduction of 11 per cent in the flow. The average duration of the oxygen administration was 8 minutes. The absence of a fall in blood pressure makes it unlikely that the animal was partially anoxic. In four experiments there was an average

reduction in flow of 10.5 per cent in spite of a 10 per cent increase in blood pressure. There was no significant change in heart rate such as reported by Whitehorn et al. (25) and Dripps and Comroe (4) who found a decrease in pulse rate in normal males breathing 100 per cent oxygen. Also of note is that the degree of the fall in coronary flow is almost identical with the decrease in cerebral

TABLE 5

*Relationship of coronary flow and cardiac output (C.O.) under conditions of anoxia and shock*

EXPT. NO.	TIME	MEAN ART. B.P.	FLOW CC/100G/MIN	% C.O.	C.O.	REMARKS
Anoxia						
55	11:40	115	51.4	5.3	970	Control
55	11:55	115	71.6	9.2	780	10% O <sub>2</sub> 6 min.
58	12:50	85	77.6	15.4	500	Control shock acidosis
58	12:57	51	76.5	26.8	282	10% O <sub>2</sub> 5 min.
Shock						
53	11:30	83	47.3	5.4	785	Control
53	12:30	48	55.3	9.7	515	
62	11:30	120	65.3	7.3	875	Control
62	13:05	65	56.2	13.3	410	

TABLE 6

*Effect of oxygen content upon coronary flow*

MEAN ART. B.P.		% CHANGE	CORONARY FLOW CC/100G/MIN.		% CHANGE	TIME*
Before	After		Before	After		
100% oxygen						
No. experiments 6						
112	112	0	55.85	49.75	-11	8 min.
No. experiments 4						
89	98	+10	53.00	47.50	-10.5	11 min.
10% oxygen						
No. experiments 6						
91	101	+11	47.70	77.95	+64	4 min.

\* Duration of oxygen administration.

flow during the inhalation of 100 per cent oxygen as reported by Dumke and Schmidt (5) in monkeys and Kety and Schmidt (20) in man.

Anoxia proved to be a potent coronary dilator as has been shown by other investigators (11, 18). In 6 experiments during which a 10 per cent oxygen mixture was administered from a Douglas bag, the average increase in coronary



flow was 64 per cent with an accompanying rise in blood pressure of 11 per cent (table 6). This increase in coronary flow is greater than the 37 per cent increase in cerebral blood flow which Kety and Schmidt (20) found to occur in men breathing 10 per cent oxygen. In view of the tendency of coronary flow to vary directly with the pulse rate (see table 3), the tachycardia of anoxemia may be held responsible for some of this difference. The general tendency is the same.

C. *The effect of carbon dioxide.* Hilton and Eichholtz (18) and Markwalder and Starling (22) found that there was a dilatation of the coronary arteries with the administration of carbon dioxide. Green (11) was unable to find any significant alteration of coronary flow with concentrations of carbon dioxide up to

TABLE 7  
*Effect of carbon dioxide upon coronary flow*

MEAN ART. B.P.			FLOW CC/100G/MIN.		AVG. CO <sub>2</sub> CHANGE	TIME*
Before	After	% Change	Before	After		
7% CO <sub>2</sub> in room air—6 experiments						
104	96	-7.5	23.33	23.20	+6.2 vol.%	5 min.

\* Duration of CO<sub>2</sub> administration.

TABLE 8  
*Effect of low blood pH upon coronary flow*

MEAN ART. B.P.			FLOW cc/100g/MIN.			CARDIAC OUTPUT		pH	
Before	After	Change	Before	After	Change	Before	After	Before	After
		%			%				
112	98	-13.6	62.7	63.2	+1.0	1076	765	7.35	7.08

8 per cent. Our findings agree with the latter author. In 6 experiments during which 5 and 7 per cent carbon dioxide in room air was administered from a Douglas bag, we could find no consistent alteration of coronary flow, even though the coronary artery carbon dioxide content rose an average of 6.2 vol. per cent (table 7). In this respect the findings are different from the average increase of 75 per cent in the cerebral blood flow of man during the inhalation of 5-7 per cent carbon dioxide reported by Kety and Schmidt (20). They are however similar to corresponding effects on total cerebral blood flow in the monkey (5, 23).

D. *The effect of pH changes.* A lowering of the blood pH was found to have an unexpectedly great effect upon the coronary blood flow. To reproduce low blood pH experimentally, 0.1 N HCl was given slowly by intravenous infusion to 5 dogs. The average results of these experiments are shown in table 8. It

is noted that even though there was a drop in blood pressure of nearly 14 per cent, there was no decrease in the average coronary flow.

This change in flow with the lowering of pH is of the same magnitude as reported by Hilton and Eichholtz using the heart-lung preparation (18).

*Comment.* The findings reported in the preceding sections show that the coronary circulation of the dog resembles the cerebral circulation of man as far as the effects of alterations of  $pO_2$  and pH are concerned (20). The main difference appears to be a much reduced sensitivity of the dog's coronary arteries to changes in  $pCO_2$ , but this is compensated by a greater sensitivity to changes in pH.

The results are also in agreement with the concept of the redistribution of the blood in peripheral vascular collapse. Under conditions when the cardiac output falls as it does in shock the relative amount of the blood being supplied to the heart is increased. With acceleration of pulse rate, coronary flow increases. When oxygen tension in the arterial blood falls or the hydrogen ion concentration decreases, more blood goes to the heart. Our data also indicate that the coronary arteries are not able to participate in generalized vasoconstriction. Apparently many factors can operate to insure a good supply of blood to the heart in time of circulatory stress.

The frequent findings during the course of these experiments which suggested an intrinsic control over the amount of blood flowing through the coronary arteries have caused us to turn our attention to the questions of oxygen consumption, cardiac metabolism, and cardiac work. These studies are now in progress and will be reported at a later date.

#### SUMMARY

1. In the anesthetized dog breathing spontaneously normal coronary flow has been found to be of the order of 65 cc. per hundred grams of heart per minute.

2. Coronary flow was found to bear a close relationship to blood pressure: a given percentile drop in the latter led to a similar percentile drop in the former. An acceleration of the pulse rate led to an increased coronary flow.

3. No significant coronary vasoconstrictor activity of the vagus or accelerator nerves could be found. The dilator qualities of epinephrine and acetylcholine are discussed.

4. The fraction of the cardiac output flowing through the coronary arteries varied inversely with the cardiac output. The heart of these animals under "normal" conditions received 4-5 per cent of the total cardiac output but this proportion was much higher when the circulation was depressed.

5. Anoxia and a low blood pH increased coronary flow but carbon dioxide was without significant effect.

The authors wish to express their appreciation to Dr. Carl F. Schmidt without whose advice and guidance these experiments would not have materialized. They also wish to acknowledge the assistance of Doctors Detwiler and Blumner with some of the experiments.

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# THE METABOLISM OF GLUCOSE IN STARVATION AND WATER DEPRIVATION<sup>1</sup>

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The ability of the organism to metabolize glucose is profoundly affected by the antecedent diet. Starvation and severe restriction of the carbohydrate intake diminish the tolerance to glucose. Glycosuria, a diabetic blood sugar curve, and absence of the normal rise of the respiratory quotient (R.Q.) mark the response of the fasting animal or subject to administered glucose. The extensive literature bearing on this phenomenon, appropriately named "starvation diabetes," has been comprehensively reviewed by Chambers (1) and more recently discussed by Peters (2). The metabolic defect of this condition is thought to be one of carbohydrate oxidation and not of hepatic glycogenesis as was formerly believed. The evidence rests mainly on the behavior of the R.Q., which, however, because of its composite nature, does not lend itself to unequivocal interpretation.

In contrast to the abundance of information on the blood sugar reaction to glucose in fasting and undernutrition, little is known about intermediary carbohydrate metabolism under these conditions. The finding of Bueding, Stein, and Wortis (3) that the pyruvate concentration in the blood increases following glucose administration due to the breakdown of the sugar, provides a means, independent of the R. Q. response, to estimate *in vivo* the intensity of an intermediate stage of glucose oxidation. In the first series of experiments to be reported here, the influence of starvation on the oxidation of administered glucose was studied by observing, in addition to the responses of the blood and urine sugar and, in a few cases, of the R.Q., the changes in blood pyruvate and its reduction product, lactate, and also, as further indications of the degree of glucose utilization, the changes in plasma and urinary inorganic phosphate. The results conform with the conclusions drawn from earlier studies of the respiratory exchange, indicating that fasting causes a temporary impairment of the ability to oxidize glucose.

In the second series of the present experiments, the influence on glucose metabolism of severe curtailment of both food and water intake, such as might be imposed upon castaways, was investigated in a similar manner. Various, and in part contradictory, findings have been reported concerning the effects of

<sup>1</sup> This study was a part of work done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Massachusetts General Hospital, and also was supported in part by a grant from Swift and Company, Chicago.

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water depletion on carbohydrate metabolism, particularly in connection with the effects of starvation. Thus it has been claimed that experimental dehydration of fasting infants prolongs the diabetic-like hyperglycemia following glucose and precipitates the development of fasting ketosis (4). The formation and storage of liver glycogen, which requires considerable quantities of water, has been reported to be impaired in dehydration (4, 5). MacKay et al. (6), on the other hand, have concluded, on the basis of experiments with fasting rats, that withdrawal of water alleviates hunger ketosis and replenishes the liver glycogen stores. Insulin sensitivity has been observed to be both increased (7) and decreased (8) in dogs by dehydration, and unchanged in infants (4) and chicks (9) by dehydration plus starvation. The results of the present study indicate

TABLE 1  
*Daily dietary regimen previous to glucose tolerance tests*

SUBJECTS	DAYS	DIET	PRO- TEIN	CARBO- HY- DRATE	FAT	WATER	CALO- RIES
			gm.	gm.	gm.	cc.	
E. B., M. G., W. H., P. J.	6	1000 cc. water *	0	0	0	1000*	0
R. E., T. H.,† W. H.,† P. J.	6	50 gm. glucose 50 gm. pork fat 400 cc. water‡§	0	50	45	405‡§	606
R. E., T. H.,† W. H., P. J.†	8	50 gm. glucose 50 gm. pork fat 400 cc. water‡	0	50	45	405‡	606
T. H.,† P. J.	8	150 gm. caramel 400 cc. water‡	1.4	105	29	414‡	685

\* E. B. received in addition 200 cc. water, M. G. 4.5 grams NaCl.

† Received 5 mgm. thiamine hydrochloride daily.

‡ One hundred cubic centimeters water on the first day.

§ W. H. and P. J. received in addition 200 cc. sea water beginning the second day.

that when large amounts of glucose are suddenly ingested by individuals in a state of semi-starvation and dehydration, breakdown of the sugar proceeds, after an initial lag, at a rapid rate and may continue with unabated intensity until marked hypoglycemia ensues.

PROCEDURE. Six healthy young men, conscientious objectors who had been transferred to the Hospital as volunteers from Civilian Public Service Camps, were subjected repeatedly to complete or partial food and water deprivation for periods lasting 6 and 8 days. The composition and amount of food and fluid ingested during these periods are listed in table 1. Two types of dietary regimens were followed: *a*, a regimen of complete starvation during which merely a liter of water was ingested daily, and *b*, a regimen of partial food and water deprivation, allowing for 606 or 685 calories of sugar-fat mixtures and 400 cc. water per day.

For reasons to be specified below, 5 mgm. thiamine hydrochloride were supplied daily in 5 of the 10 experiments with the low calorie-low water diets. Sodium chloride or ocean water was supplied in several experiments for reasons with which this study is not directly concerned.

On the morning terminating the experimental periods, 1.75 grams dextrose per kilogram body weight were administered orally, the customary procedure of glucose tolerance tests being followed. The sugar was given as a 25 per cent solution after complete starvation and as an 8 per cent solution after partial starvation and water deprivation. Thirty cubic centimeters fresh lemon juice were added to make the solutions more palatable. Venous blood samples were taken, with least possible stasis, in the basal state and at approximately hourly intervals following the administration of glucose. The samples were analyzed for their content of glucose (10), pyruvic acid (11)<sup>3</sup>, lactic acid (12), and, following complete starvation, also for the inorganic phosphorus content of the plasma (13). For the determination of pyruvic and lactic acids, blood was collected without delay in flasks containing oxalate, iodoacetate, and fluoride, and the protein precipitated in 4 volumes of cold 10 per cent trichloroacetic acid. Control data were secured from 4 subjects after complete recovery from the experiments.

In three of the tests taken after starvation, urine samples were collected immediately before and approximately 2½ and 4 hours after glucose intake and analyzed for sugar (14) and phosphate (13). In two of these tests and in a control, the respiratory exchange was measured. Expired air was collected during 10 minute periods in a Tissot gasometer and analyzed with the Haldane apparatus.<sup>4</sup> The nitrogen content in the urine samples was determined for the calculation of the non-protein R.Q.s.

**RESULTS.** The changes in the concentration of blood glucose, pyruvic acid, lactic acid, and of plasma phosphate following ingestion of glucose in complete starvation, partial starvation-dehydration, and in the normal postabsorptive state are summarized graphically in figure 1. Three sets of composite curves represent the average results obtained in each of the three dietary states.

*Effects of fasting on the blood glucose, pyruvate, lactate, and plasma phosphate responses to glucose.* Figure 1 shows that the average blood sugar curve of the fasting subjects follows a course characteristic of starvation diabetes. The basal level, 80 mgm. per cent, is slightly below that of the controls. After glucose ingestion the blood sugar rises at once steeply and continuously for two hours to reach a peak of 250 mgm. per cent. Four hours later it was still as high as 186 mgm. per cent.

The fall in the concentration of the plasma inorganic phosphate, which usually follows administration of carbohydrates, is less rapid and more protracted as a result of fasting. During the first hour the average plasma phos-

<sup>3</sup> Ethyl acetate was used as solvent for the extraction of the dinitrophenylhydrazone of pyruvic acid.

<sup>4</sup> We are greatly indebted to Miss Mary Lennan, Miss Audrey Dennison, and Ensign Louis J. Pecora (USN) for taking the respiratory measurements.

phorus decreases by only 0.41 mgm. per cent as compared to a decrease of 0.78 mgm. per cent in the control tests. A similar slow and prolonged decline in plasma phosphate following glucose administration was observed by Goldblatt and Ellis (15) in a subject after a 39 hour fast.

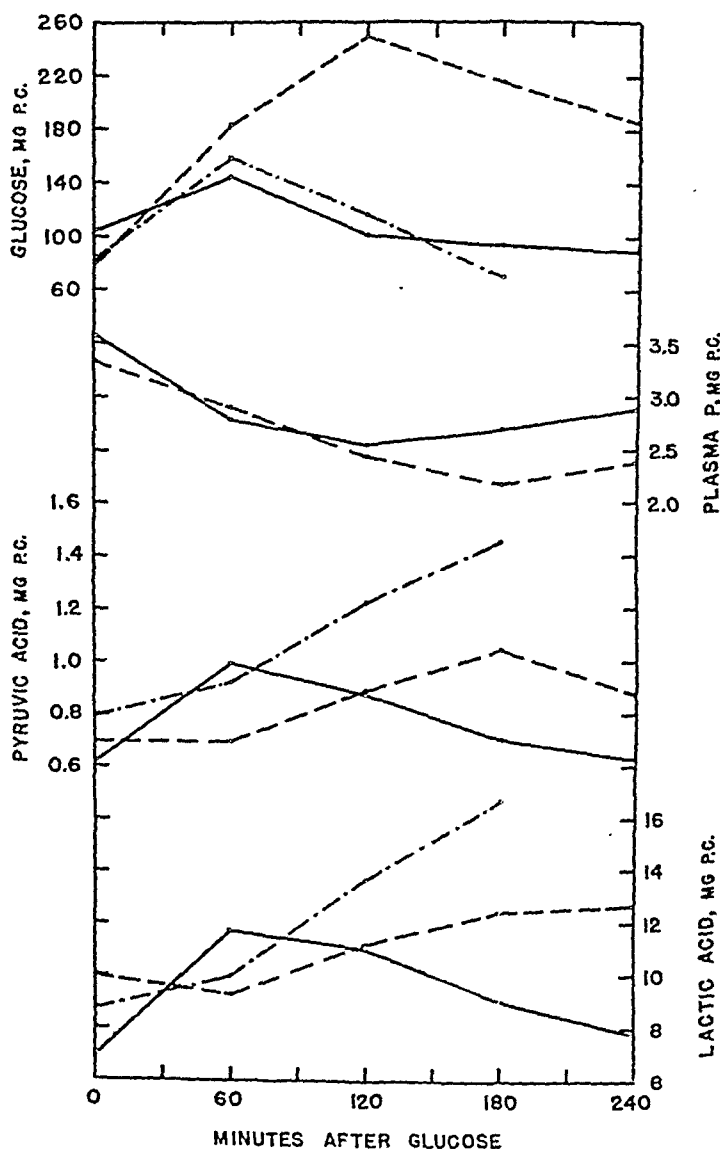


Fig. 1. Mean levels of blood glucose, pyruvic acid, lactic acid, and plasma inorganic phosphorus before and after ingestion of glucose.

— normal postabsorptive state (4 expts. on 4 subjects);

--- 7th day of fast (4 expts. on 4 subjects);

- - - - 7th or 9th day of partial food and water deprivation (10 expts. on 4 subjects).

The pyruvic and lactic acid content of the blood, which normally increases shortly after glucose administration, remains unchanged or even decreases slightly at first in the fasting subjects. Since at the same time glucose accumulates in the blood at an abnormally rapid rate, the absence of the rise in pyruvic

and lactic acids can hardly be the result of an increased rate of removal, but rather signifies a failure in the formation of these two acids from the ingested glucose.

After a delay of about one hour the concentrations of pyruvic and lactic acids begin to rise slowly above the basal levels. The usual maxima of 1 and 12 mgm. per cent are not attained, on the average, until the end of the third hour. Pyruvic acid then starts to disappear from the blood, while the lactic acid concentration remains at the peak level for another hour. No other instance of a major discrepancy between changes in pyruvate and lactate was noted in the 18 experiments reported. The lactate/pyruvate ratios remained otherwise more or less fixed during a given test at values ranging from 10 to 15.

*Urinary phosphate excretion following glucose administration in fasting.* It is well known that glucose administration to a normal animal or subject causes

TABLE 2  
*Urinary excretion of sugar and phosphate following glucose administration on the 7th day of fasting*

SUBJECT	MIN. BEFORE AND AFTER GLUCOSE	URINE VOL.	SUGAR	PHOSPHORUS
		<i>cc./hour</i>	<i>mgm./hour</i>	<i>mgm./hour</i>
M. G.	-117 to 0	31.8	0	23.4
	0 to 166	38.3	725	22.4
	166 to 274	60.0	1810	6.4
W. H.	-190 to 0	16.4	0	21.9
	0 to 155	65.4	704	46.4
	155 to 248	117.7	526	14.3
P. J.	-200 to 0	20.4	0	22.4
	0 to 152	35.5	781	27.7
	152 to 260	119.0	700	20.2

prompt retention of inorganic phosphate. However, this is not the case in fasting (table 2). The usual decline in the rate of urinary phosphate excretion is either postponed for more than 2 hours (subjects M. G. and W. H.) or is practically absent for the entire 4 hour test period (P. J.). There may be even an initial increase in the rate of phosphate output.

The failure to preserve inorganic phosphate after glucose administration is paralleled by the familiar glycosuria of starvation diabetes (table 2). Together with the slow disappearance of phosphate from the plasma, the undiminished urinary phosphate loss suggests a temporary impairment of phosphorylation reactions. A decreased ability of the liver to form adenylypyrophosphate, which is required for the phosphorylation of glucose, has been suggested by Kaplan and Greenberg (16) as an important factor responsible for the decreased glucose tolerance of fasting.

*Effect of food and water restriction on the blood glucose tolerance curve.* Since



both the 6 and 8 day sugar-pork fat and the 8 day caramel diets had practically the same effect on the outcome of the glucose tolerance tests, only the average results of the 10 low calorie-low water experiments have been recorded in figure 1. This figure shows that the usual course of the blood glucose tolerance curve is altered to some extent by 6 and 8 days of food and fluid restriction. The glucose concentration in the basal venous samples, averaging 84 mgm. per cent, is, as on the seventh day of fasting, somewhat lower than that of the controls. During the first hour following glucose ingestion it rises to 159 mgm. per cent, slightly exceeding the control figure of 145. The curve then declines in the usual way, but without leveling off at the end of the second hour. Instead, it continues its downward course during the third hour, the 180 minute blood glucose being 71 mgm. per cent. Thus both the initial rise and subsequent fall of the blood glucose curve become exaggerated after a week of restricted food and water intake.

Actually the posthyperglycemic hypoglycemia was considerably more pronounced than can be gathered from figure 1. In this figure the curve has not been drawn beyond the third hour, since the majority of the experiments were discontinued before another blood sample was taken. The appearance during the fourth hour of typical signs of marked hypoglycemia made it advisable to terminate the experiments and supply the subjects with sugar. One subject, P. J., was particularly susceptible to the hypoglycemic reaction. In one glucose tolerance test, taken on the seventh day of the glucose-pork fat regimen, a blood sugar of 41 mgm. per cent was observed 183 minutes after the ingestion of glucose. Ten minutes later P. J. was on the verge of losing consciousness. In a second test, taken on the ninth day of the caramel regimen, he had a 213 minute blood sugar of 43 mgm. per cent and began to faint during the 223rd minute. On the other hand, this subject did not experience the slightest discomfort during the control test, and when given glucose on the seventh day of fasting, had a 205 minute blood sugar of 208 mgm. per cent.

*Effect of food and water restriction on the blood pyruvate and lactate response to glucose.* Abnormal pyruvate and lactate curves characterize the reaction of the underfed and dehydrated subjects to glucose (fig. 1). During the first hour following glucose injection the two metabolites increase only little in the venous blood. Throughout the second and third hours, which normally bring a return to the starting levels, they accumulate quite rapidly and continuously. The average 3 hour values are 1.45 and 16.7 mgm. per cent as compared to the control maxima (at 1 hr.) of 0.99 and 11.7. Since the prolonged and exaggerated rise in blood pyruvate and lactate is accompanied by an equally prolonged and exaggerated fall in blood glucose, it is probably the result of an increase in pyruvate and lactate formation from the ingested glucose rather than of an impairment of their removal.

Because of the onset of hypoglycemia during the fourth hour, 4-hour blood samples were taken in only three tests. The analyses disclosed a slight decline in the concentrations of pyruvate and lactate during that period.

*Comparison of the various blood responses to glucose with the response of the R.Q.*

A set of data on an individual subject, W. H., showing his response to glucose ingestion in the normal basal state, on the seventh day of complete starvation, and on the ninth day of severe curtailment of the food and water intake is pre-

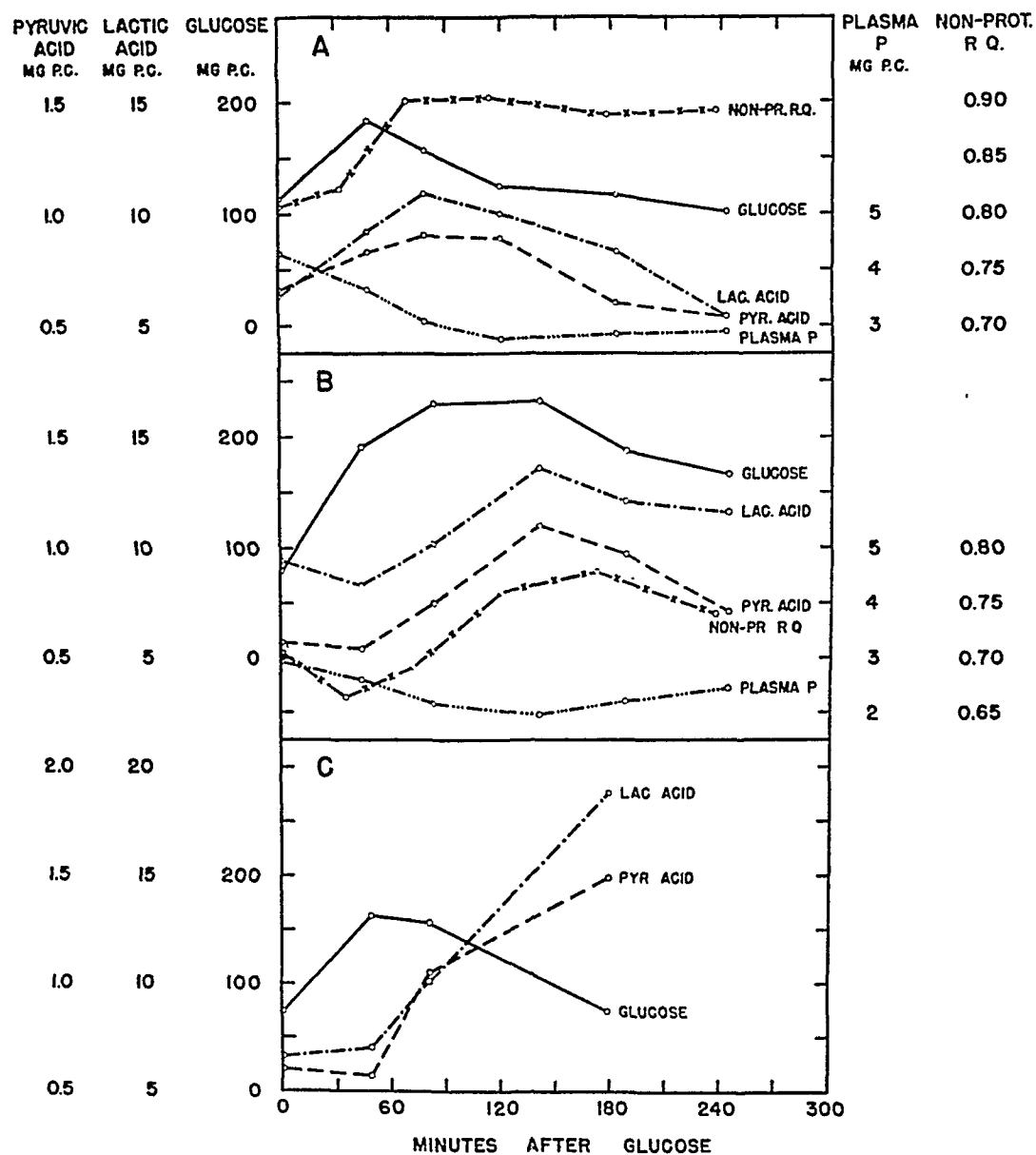


Fig. 2. Subject W. H. Changes in blood glucose, pyruvic acid, lactic acid, plasma inorganic phosphorus, and non-protein R.Q. following ingestion of glucose. A: normal postabsorptive state; B: 7th day of fasting plus 1 liter water; C: 9th day of 50 grams glucose, 50 grams pork fat, 400 cc. water.

sented in figure 2. Here the results of each test have been grouped together. As in figure 1, the alterations in the metabolism of glucose produced by fasting and by underfeeding and dehydration are clearly apparent.

In addition to the blood responses, the respiratory exchange of this subject was determined in the fasting and control experiments. Figure 2 shows that the

non-protein R.Q., which in the control test rose from 0.81 to 0.90 soon after glucose intake, declined at first on the seventh day of fasting and rose later on to 0.78. This is a significant rise, although the quotient remained within a range which is customarily interpreted as reflecting a predominance of fat over carbohydrate combustion. A similar observation was made on a second fasting subject, M. G., whose non-protein R.Q., after an initial decrease from 0.66 to 0.63, increased to 0.78  $3\frac{1}{2}$  hours following glucose ingestion.

The fact that in starvation the R.Q. remains at relatively low levels following the administration of glucose has been taken to indicate an incomplete oxidation of the sugar (15, 17). Root, Stotz and Carpenter (18) found that a negative response of the R.Q. in diabetics after the ingestion of glucose or fructose was always accompanied by a negative response of the blood pyruvic and lactic acids. In the present experiment (fig. 2, B) both the R.Q. and the two blood acids failed to rise immediately after the administration of glucose. The

TABLE 3

*Effect of thiamine supplementation on the blood glucose, pyruvate, and lactate responses to glucose on the 7th or 9th day of restricted food and water intake*

Five milligrams thiamine hydrochloride per day. Both the experimental and control figures represent the mean results of 5 experiments on 3 subjects. The results are expressed in milligrams per cent.

BLOOD CONSTITUENT	SUBJECTS	HOURS AFTER GLUCOSE			
		0	1	2	3
Glucose	Control	82	159	115	69
	Experimental	86	160	119	74
Pyruvic acid	Control	0.80	0.92	1.25	1.43
	Experimental	0.79	0.94	1.27	1.48
Lactic acid	Control	8.6	9.8	13.5	16.3
	Experimental	9.0	10.2	14.0	17.1

starved subject was unable to oxidize the ingested sugar at a detectable rate. After a considerable delay, the concentration of the two acids increased to an even slightly greater extent than in the control experiment, while the R.Q. did not rise above 0.78. Suppression of a reaction with a high R.Q., such as conversion of glucose into fat, or stimulation of a reaction with a low R.Q., such as conversion of fat into carbohydrate, may have been responsible for the failure of the R.Q. to reach higher levels.

*Effect of thiamine supplementation on the blood responses to glucose after food and water restriction. Urinary thiamine excretion in starvation and dehydration.* The possibility that inadequate removal of pyruvic and lactic acid may have been a factor contributing to their abnormal increase in the blood following glucose ingestion after partial starvation and water deprivation cannot be excluded with certainty. Bueding, Stein and Wortis (3) have shown that excessive accumulation of pyruvic acid in the blood, resulting from its diminished breakdown,

characterizes the response of thiamine deficient subjects to glucose. In view of persistent, though repeatedly refuted reports in the literature (19; and others) that manifestations of thiamine deficiency may appear within a few days after lowering the intake of the vitamin to inadequate levels, 5 mgm. of thiamine hydrochloride were added to the daily food ration in five of the ten experiments

TABLE 4

*Urinary excretion of thiamine during periods of starvation and water deprivation*

SUBJECT	DAILY DIET	24 HOUR URINARY EXCRETION			
		Day	Vol.	N <sub>2</sub>	Thiamine
			cc.	grams	$\gamma$
E. B.	None	1	815	9.32	50
		2	555	9.77	25
		3	560	11.99	14
		4	510	11.63	8
M. G.	600 cc. water*	1	875	8.61	90
		3	870	11.94	34
		6	680	11.32	0
M. G.	1000 cc. 0.9% NaCl	1	920	9.67	51
		3	1305	14.18	20
		6	910	9.46	2
R. E.	600 cc. 1% NaCl 150 gm. Caramel†	1	585	10.07	52
		3	510	7.37	10
		6	460	5.50	6
P. J.	400 cc. water*	1	750	9.06	70
	200 cc. sea water	3	530	10.66	6
	50 gm. glucose	5	570	8.64	10
	50 gm. pork fat‡	8	410	5.37	5
R. E.	400 cc. water*	1	900	8.55	41
	50 gm. glucose	3	302	6.90	12
	50 gm. pork fat‡	5	270	6.37	6
		8	250	5.74	7

\* On the 1st day only 100 cc.

† Contained 8 $\gamma$  thiamine.

‡ Contained no thiamine.

with the low calorie-low water diets, in order to ascertain whether or not the lack of this vitamin in these diets<sup>5</sup> was a factor in the abnormal elevation in blood pyruvate and lactate. The subjects were matched in such a way as to make thiamine intake the sole experimental variable (cf. table 1). The results of these experiments are summarized in table 3. It is evident that addition of thiamine to the diet had no effect on the response to glucose. Both the group

<sup>5</sup> The glucose-pork fat diets contained no thiamine, the caramel diet 8  $\gamma$  per 150 grams.

receiving thiamine and the control group exhibit the same pronounced elevations in blood pyruvate and lactate and the same marked fall in blood sugar.

In connection with these experiments, the course of the urinary excretion of thiamine was followed over periods of complete and partial starvation and water deprivation. Since very few data exist in the literature on urinary thiamine loss under these conditions, we take the opportunity to present some typical results (table 4). The 24 hour urinary thiamine content was determined by a thiochrome procedure (20) with a photoelectric fluorophotometer.

Table 4 demonstrates that thiamine excretion, already quite low on the first day of the various starvation regimens, declines very rapidly to zero or near zero values after 4 to 6 days. There appears to be a lack of correlation between thiamine output and urine volume. On the other hand, a comparison of the third day figures shows that the decrease in urinary thiamine is somewhat less rapid in complete than in partial starvation. The difference, if significant, is probably due to an accelerated breakdown of body tissue in complete fasting (cf. nitrogen excretion column in table 4), which liberated larger amounts of endogenous thiamine than the protein-sparing carbohydrate-fat mixtures. Nevertheless, the fall in the thiamine output of the fasting subjects is much more precipitous than was observed by Perlzweig et al. (21) in healthy subjects during a four day fast. Possibly the thiamine reserves of our subjects, who during the intervals between the experiments were living on a hospital orderly diet, were lower than those of Perlzweig's subjects.

**DISCUSSION.** *Effects of complete starvation.* The delayed and sluggish rises in blood pyruvate and lactate, which characterize the response to glucose ingestion after nearly a week of fasting, were taken to indicate, in view of the simultaneous excessive hyperglycemia, an impairment in the formation of these two products of intermediary glucose metabolism. The organism, after having been prevented for a time from metabolizing carbohydrates as a main source of energy, appears to require a certain latent period to get the oxidation of administered glucose started. This latent period coincides with the initial steep rise in the blood sugar concentration, and could mean that after starvation a high degree of hyperglycemia has to be attained before carbohydrate combustion is initiated or intensified.

The retarded rise of the R.Q., the slow rate of decrease of the plasma phosphate content, and the delay in the decline of the urinary phosphate excretion are responses of the starved individual to glucose which, in association with the negative responses of the blood pyruvate and lactate, can best be interpreted as additional manifestations of a temporary inhibition of glucose oxidation. Similar deviations from normal carbohydrate metabolism have been encountered in diabetes mellitus. After glucose administration to diabetic humans or animals the fall in blood inorganic phosphate is likewise abnormally slow and protracted or entirely absent (22), and urinary phosphate excretion continues at an undiminished rate (23); blood pyruvate (24-26) and lactate (26-28) fail to rise in the usual manner, signifying, particularly in conjunction with a simultaneous negative R.Q. response (18), a deficiency in carbohydrate oxidation. Thus the

results of the present experiments extend the parallelism between the phenomena of diabetes mellitus and starvation diabetes and underline the common nature of the deficient response to glucose in these two conditions.

*Effects of partial starvation and water deprivation.* Since partial starvation was associated in every instance with dehydration, the experiments leave the question unsolved whether the hypoglycemic reaction to glucose was due to food or to water deficiency or to a combination of both. Depletion of the hepatic glycogen stores, which in all likelihood resulted from underfeeding, may have made a defense against hypoglycemia by hepatic glycogenolysis difficult. That the prolonged decline in blood sugar, however, was not primarily a result of insufficient release of glucose by the liver, but of increased glucose oxidation, is evidenced by the fact that it was accompanied by an abnormal accumulation in the blood of the two breakdown products of glucose, pyruvic and lactic acids. On the other hand, the immediate and rapid rise in blood sugar following glucose ingestion makes it seem unlikely that the initial negative pyruvate and lactate responses were caused either by increased removal of these compounds from the blood or by impaired absorption of the ingested sugar into the blood stream or by its retention in the liver, but suggests that they were due to deficient glucose oxidation.

The daily intake of small, but not negligible amounts of carbohydrate was probably essential for the elicitation of the hypoglycemic reaction. These amounts were sufficient to prevent starvation ketosis, as evidenced by a low renal output of organic acids and ammonium (cf. 29). But they were not sufficient to prevent an impairment in glucose tolerance, as shown by the initial behavior of the blood pyruvate and lactate. From the data in the literature it appears that while undernutrition *per se* has little effect on the utilization of carbohydrate (1), subsistence on diets deficient in carbohydrate, though not necessarily in total caloric content, may be capable of inducing marked hypoglycemia following glucose administration, preceded, as in this study, by a somewhat exaggerated rise in blood sugar (30, 31). A glucose tolerance curve of this type, provoked by a low carbohydrate diet, has been found to be characteristic of functional hyperinsulinism (32). Teitelbaum (33) obtained such a glucose tolerance curve in an individual with functional hyperinsulinism by feeding a starvation diet low in carbohydrate. He explains the hypoglycemic response on the basis that the starvation diet lowered glucose tolerance, exaggerated the hyperglycemia subsequent to glucose intake, and thus led to an excess outpour of insulin when the insulogenic stimulus level of blood sugar was reached and passed. The hypoglycemic reaction described here may be explainable in a similar fashion, particularly if, as the finding of Andrews (7) suggests, insulin resistance was lowered by water deprivation. In any event, the occurrence of the initial negative pyruvate and lactate responses, the abruptness of their reversal (cf. fig. 2), and the subsequent unopposed fall of the blood sugar leave the impression that the normal interplay of the mechanisms concerned with the regulation of carbohydrate metabolism is seriously upset in a state of semi-starvation and dehydration.

## SUMMARY

1. The response of normal young men to glucose ingestion was studied after a 6 day fast and after 6 and 8 days of subsistence on 600-700 calories of sugar-fat mixtures plus 400 cc. water per day.

2. Fasting, in addition to producing a diabetic blood sugar curve, glycosuria, and a delay in the rise of the respiratory quotient, had the following effects: the usual rises in blood pyruvate and lactate were delayed and sluggish; the fall in plasma inorganic phosphate was slow and protracted; urinary phosphate excretion continued undiminished for 2 hours or more. The delayed pyruvate and lactate responses are taken to indicate, in conjunction with the other responses, a temporary inhibition of glucose oxidation.

3. Partial starvation and dehydration altered the normal response to glucose as follows: The rise in blood glucose was somewhat steeper than usual and was followed by a prolonged fall to alarmingly low levels. During the rise of the blood glucose curve the blood pyruvate and lactate levels remained almost stationary, but increased during the declining phase of the blood sugar curve to reach excessively high values. The hypoglycemic reaction is taken as an indication, in view of the excessive rises in blood pyruvate and lactate, of enhanced glucose oxidation uncompensated by increased gluconeogenesis.

4. Addition of thiamine to the restricted diets had no effect on the blood pyruvate, lactate, and glucose responses.

5. Data are presented on urinary thiamine loss during starvation and water deprivation.

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# EFFECTS OF HYPOTHERMIA UPON THE SPECIFIC GRAVITY AND PROTEINS OF THE BLOOD OF CHICKENS<sup>1</sup>

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Barbour, McKay and Griffith (1) reported that as body temperature in monkeys decreased, the specific gravity and proteins of the blood increased and reached a maximum at 30° C. Below this body temperature the gravities decreased, and at 23°C. they approached normal levels. When the animals at this body temperature were warmed, the serum proteins did not reverse but continued to decline significantly below normal levels. In chilled and rewarmed rats, the changes in serum proteins and chlorides followed the same pattern as in monkeys. Randall (2, 3), Sturkie (4, 5, 6), and others have reported on the various effects of hypothermia in fowl, but no one has studied the effects of hypothermia on the specific gravity and proteins of the blood of chickens. This report concerns such a study.

**METHODS.** Hypothermia was induced in laying White Leghorn hens by suspending them, up to the neck, in water of the appropriate temperature (see Sturkie 4, 5, or 6 for further details).

Specific gravities of the whole blood and plasma were determined by the copper-sulphate method of Phillips et al. (7). A series of sulphate solutions, graded at intervals of 0.001 in specific gravity, were used. Blood or plasma to be tested was obtained in a hypodermic syringe or capillary tube, and one drop at a time allowed to fall into the sulphate solution. On entering the solution, each drop becomes encased in a sack of copper-proteinate, and its rise or fall within 15 to 20 seconds reveals its gravity relative to that of the solution. According to these workers, the gravities thus obtained on human blood are accurate to within  $\pm 0.0002$  to  $0.0003$ . They used the following equation in estimating plasma proteins from specific gravity of plasma: Plasma proteins = 360 (Gravity of Plasma—1.0070). This equation was used in estimating the plasma proteins of chicken blood. The reliability of the equation for chicken blood was not determined; however, the protein values obtained (average 4.3 grams per cent) apparently are not out of line with those obtained by direct methods (8, 9).

**RESULTS.** The first sample of blood of the hens was taken when the body temperature was normal, and the specific gravities of the whole blood or plasma were determined. Hypothermia was then induced, and later second blood samples were taken and the specific gravities determined. The results are shown in table 1. The specific gravities of the whole blood of 19 hens whose body temperatures varied from 36.1 to 26.7°C., when the second blood sample was obtained, averaged 1.04823, compared to 1.04495 for the first sample. There

<sup>1</sup> Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey, Department of Poultry Husbandry.

was an increase in 18 of the second blood samples, and the mean increase is highly significant (*t* value, 4.19). Ten of these birds were then warmed for periods of 1 to 5 hours. Their body temperatures ranged from 41.1 to 33.3°C.

TABLE 1

*Effects of hypothermia upon specific gravity and proteins of blood of hens*

FIRST SAMPLE			SECOND SAMPLE						THIRD SAMPLE		
Specific gravity		Plasma proteins	Specific gravity		Plasma proteins	*	Body temp.	Time†	Specific gravity whole blood	Body temp.	Time
Whole blood	Plasma		Whole blood	Plasma							
		<i>gms.</i>			<i>gms.</i>		°C.	<i>hours</i>		°C.	<i>hours</i>
1.0437			1.0437				32.2	1½			
1.0390			1.0430				28.9	2			
1.0440			1.0495				31.7	1	1.0440	41.1	5
1.0490			1.0530				33.3	1½	1.0520	38.9	3
1.0470			1.0505				31.1	1¼	1.0485	33.3	1½
1.0510			1.0545				31.7	1¼	1.0495	34.4	3
1.0455			1.0463				32.8	1	1.0410	37.2	3
1.0365			1.0410				33.3	1	1.0380	35.6	½
1.0475	1.0208	4.97	1.0495	1.0225	5.58	0.61	28.9	2	1.0465	40.6	5
1.0490	1.0197	4.57	1.0545	1.0225	5.58	1.01	27.8	2	1.0486	39.4	5
1.0485	1.0190	4.32	1.0550	1.0215	5.22	0.90	26.7	1¼	1.0510	35.0	1
1.0492	1.0200	4.68	1.0540	1.0210	5.04	0.36	26.7	2½	1.0485	35.6	1
1.0392	1.0210	5.04	1.0410	1.0215	5.22	0.18	36.1	½			
1.0420	1.0190	4.32	1.0445	1.0190	4.32	0.0	31.1	2			
1.0455	1.0175	3.78	1.0470	1.0185	4.14	0.36	34.4	1			
1.0455	1.0190	4.32	1.0470	1.0180	3.96	-0.36	36.1	1			
1.0410	1.0180	3.96	1.0455	1.0190	4.36	0.40	33.3	1			
1.0500	1.0200	4.68	1.0515	1.0200	4.68	0.0	32.8	1½			
1.0410	1.0180	3.96	1.0455	1.0195	4.50	0.54	28.3	2½			
	1.0200	4.68		1.0210	5.04	0.36	36.1	½			
	1.0183	4.06		1.0200	4.68	0.62	23.9	6¼			
	1.0180	3.96		1.0192	4.39	0.43	24.4	2			
	1.0187	4.21		1.0203	4.78	0.57	24.4	1½			
	1.0187	4.21		1.0200	4.68	0.47	24.4	2½			
	1.0183	4.06		1.0196	4.53	0.47	25.0	1			
	1.0190	4.32		1.0205	4.85	0.53	25.0	5			
	1.0175	3.78		1.0185	4.14	0.36	25.6	2¼			
	1.0202	4.75		1.0230	5.76	1.01	25.5	1			
	1.0187	4.21		1.0217	5.29	1.08	26.1	1¼			
M 1.04495	1.01904	4.308	1.04823	1.020323	4.797						

\* Change over first sample.

† Duration of treatment.

when the third blood sample was taken and the specific gravity of the whole blood was determined. In every case the gravities were lower than those of the second samples, and in 8 cases equal to or slightly lower than the readings for the first samples.

Specific gravities of plasma were determined on 21 hens before and after

hypothermia. The duration of the low temperature treatments for the hens ranged from  $\frac{1}{2}$  to  $6\frac{1}{4}$  hours, and the body temperatures at the termination of the treatments varied from 36.1 to 23.9°C. The calculated plasma proteins before hypothermia averaged 4.308 grams per cent, and after hypothermia, 4.797. This difference represents an increase of approximately 11 per cent and is highly significant statistically (*t* value greater than 4). Nineteen of the blood samples taken after hypothermia showed increases in proteins, and the degree of the increases was independent of the degree and duration of the hypothermia.

DISCUSSION. This study indicates that the specific gravity and plasma proteins of the blood of chickens increased with lowered body temperature from 36.1 to 23.9°C., and the degree of the increase was independent of the degree and duration of the hypothermia. The body temperature of 8 of the hens when the second blood sample was taken ranged from 23.9 to 25.5°C., or 0.5 to 2°C. above the lethal temperature (Sturkie, 6). At these temperatures, the reflex responses to cold were almost completely abolished, and the birds were in a state of torpor. In most of these hens, the duration of hypothermia was  $1\frac{1}{2}$  to 2 hours, but in two of them, it amounted to 5 and  $6\frac{1}{4}$  hours. These results are contrary to those of Barbour et al. for monkeys and rats, in which cases the increase in proteins reached a maximum at certain degrees of hypothermia, but then decreased and approached normal levels when body temperature was further depressed. These workers explained the blood changes in these species as follows: "Exposure to cold with retention of protective reflexes leads to a gain in intracellular water throughout the body. When, however, the central nervous system becomes so chilled as to cause general neuro-muscular depression, the effect on the hypothalamus is to abolish the reflex responses to cold, which process includes a reversal of water shift with increased extra-cellular fluid."

Warming the birds from 1 to 7 hours in water following chilling resulted in a decrease in plasma proteins, with the values approaching normal levels, but none were significantly below normal. This finding is contrary to that of Barbour et al. in the case of rewarmed monkeys and rats. In their experiments, however, the protein levels had already declined to approximately normal levels before the animals were warmed. In none of the warmed hens had body temperatures reached normal when the plasma proteins were determined, though in some cases the temperatures were elevated as little as 2.5°C.

Warming had no effect upon specific gravity and plasma proteins of birds not previously chilled. Plasma protein values for 14 hens whose body temperatures varied from 42.8 to 46.4°C. were not significantly different from the normal values.

#### SUMMARY AND CONCLUSIONS

Hypothermia was induced in White Leghorn hens by suspending them, up to the neck, in water of the appropriate temperature. The duration of the low temperature treatment ranged from  $\frac{1}{2}$  to  $6\frac{1}{4}$  hours, and the body temperatures at the termination of the treatments varied from 36.1 to 23.9°C.

The plasma proteins, calculated from specific gravities of the plasma, before

hypothermia averaged 4.308 grams per cent, and after hypothermia, 4.797. This difference is highly significant. The degree of increase in proteins was independent of the degree and duration of the hypothermia.

Some of the birds were warmed for 1 to 5 hours following the hypothermia, when a third blood sample was taken and the specific gravities were determined. In every case the gravities were lower than those of the second sample, and in most cases equal to, or slightly lower than the readings for the first sample.

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# THE EFFECT OF RESTRICTED CALORIC INTAKE ON THE LONGEVITY OF RATS<sup>1</sup>

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McCay and co-workers (1, 2, 3, 4, 5) showed in a series of studies that animals receiving a reduced caloric intake lived considerably longer than animals fed *ad libitum*. The present study was undertaken to determine whether this beneficial effect upon the length of the life span of rats would be as pronounced on the synthetic diet used in this laboratory as on the diet used by McCay *et al.*

**EXPERIMENTAL.** A total of sixty Sprague-Dawley rats, consisting of thirty males and thirty females, were used in the experiment. All animals were kept in individual cages in an air-conditioned and temperature-controlled room for the duration of the experiment. The rats all received a basal ration consisting of sucrose 48 per cent, crude casein 36 per cent, salts IV 8 per cent, corn oil 6 per cent and 1:20 liver powder 2 per cent. The following vitamins were mixed into the casein in the amounts indicated in milligrams per 100 grams basal ration: thiamine HCl 0.72, riboflavin 0.57, nicotinic acid 0.72, pyridoxine HCl 0.72, calcium pantothenate 2.86 and choline 286. All rats received weekly two drops of an oil consisting of 50 per cent haliver oil, 48 per cent corn oil, and 2 per cent  $\alpha$ -tocopherol. Three equal groups of rats, containing half males and half females, were used simultaneously for the experiment. One group, designated the maintenance group, received the basal ration alone at a level just sufficient to allow very slow growth. One group, designated the control group received the same level of basal ration but was allowed sucrose *ad libitum*. Another group designated the restricted group received the same amount of basal ration and one-half the amount of sucrose consumed by the rats in the control group. The basal ration and the sucrose were fed in separate dishes.

At the beginning of the experiment the rats in the maintenance group were given 3.5 grams of basal ration daily. This amount supplied about 14 calories daily which McCay showed was sufficient to meet the energy requirement for limited growth of weanling rats. The basal ration was increased after five weeks on the experiment to allow slow growth of the rats in the maintenance group. The amount of basal ration fed and the amount of additional sucrose consumed by each group are shown in figure 1.

Weekly growth records were kept of all the rats. A summary of the mor-

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tality and the average growth curves is shown in figure 2. During the last 20 weeks of the experiment the food consumption and weight of the animals began to fall. There was also an increase in the number of deaths, particularly in the control and restricted groups. At the termination of the experiment four,

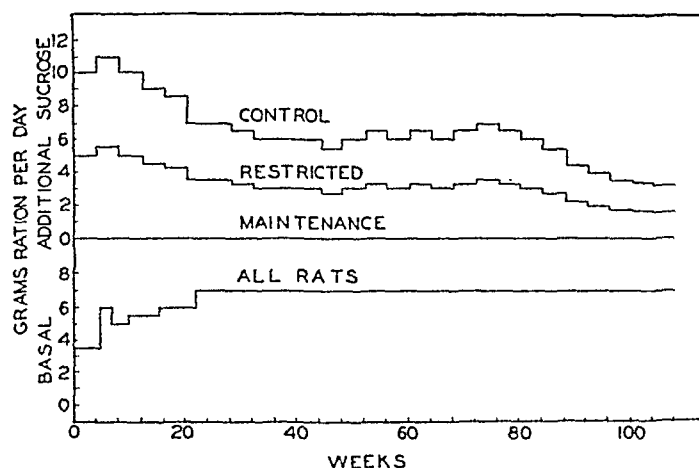


Fig. 1. Food consumption of the control, restricted and maintenance groups of rats. The number of weeks refers to the length of time after the beginning of the experiment. Weanling rats were used.

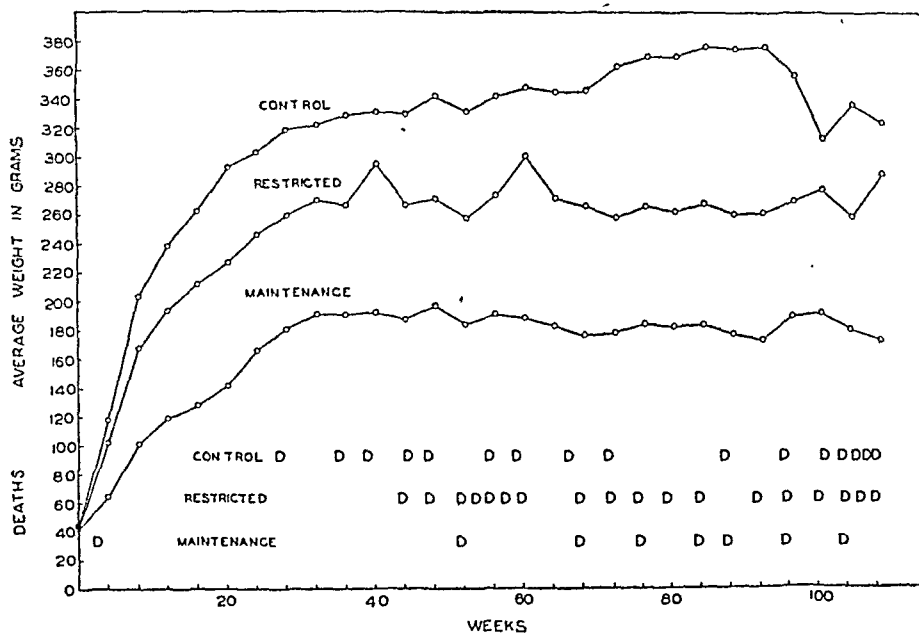


Fig. 2. Growth curves and mortality summary of the three groups of rats. The number of weeks refers to the length of time after the beginning of the experiment.

two, and twelve animals remained in the control, restricted, and maintenance groups respectively.

All animals were autopsied after death to find any gross pathological conditions in the organs and the tissues. The most striking observations in these autopsies are summarized in table 1.

DISCUSSION. The average longevity of the rat was increased in this experiment by a restriction of the caloric intake. The maintenance animals, which received the lowest caloric intake, showed the largest number of animals remaining at the termination of the experiment and the fewest number of deaths early in the life span. Although the number of animals remaining in the control and restricted groups at the termination of the experiment was approximately the same, the number of deaths occurring early in the life span was greater in the case of the control animals than in the restricted animals. There were only slight differences between the male and female animals of each group. During the last 20 weeks of the experiment the control animals began to lose weight more rapidly than the animals in the other two groups.

The sucrose consumption of the control animals was high at the beginning of the experiment. It decreased until the rats reached maturity. The level at maturity was maintained until the last few months of life span when it fell rapidly. It is interesting to note that the rats which received the greatly restricted caloric intake were able to maintain fairly normal life activity and a

TABLE 1  
*Incidence of pathological conditions*

GROUP	PATHOLOGICAL CONDITION (TOTAL NUMBER OF OCCURRENCES)		
	Tumors	Respiratory infections	Fatty degeneration of organs
Control.....	3	10	4
Restricted.....	1	12	1
Maintenance.....	0	9	0

greater life span than the controls, in spite of the drastic cut in the sucrose fed. The total caloric intake of the maintenance, control, and restricted rats during the greater part of their life span was 28, 52, and 40 calories per day, respectively. During this time all rats received 7 grams of basal ration and the control rats ate approximately 6 grams of additional sucrose ad libitum.

McCay concluded that lowered incidence of lung infections was the immediate factor responsible for the increase in longevity of rats on a restricted carbohydrate diet. The incidence of respiratory infections in our work was not significantly different in each of the three groups of rats. Gross examination of the animals showed fewer tumors and fewer fatty organs such as the liver and the kidney in the maintenance and restricted groups. Similar effects of restricted caloric intake upon incidence of tumors has been noted in rats and mice by Tannenbaum (6), Baumann (7), and Carlson and Hoelzel (8). The retarded animals were more irritable than the control animals during the last few weeks of the experiment.

Retardation of growth by a limited caloric intake increased the average longevity of the rat; however, it is difficult to attribute this increase to limited caloric intake alone. Although the ration used in this work gives good growth

and reproduction in rats it is still possible that it may not be complete in all the factors necessary for optimum health throughout the entire life span of the rat. Recent studies with the monkey by Cooperman et al. (9) have shown that unknown factors present in whole liver powder may be necessary for continuous good health of these animals.

#### SUMMARY

1. A greatly restricted caloric intake increased the longevity of the rat under the present experimental conditions although the increase was not as great on a slightly restricted carbohydrate diet as has been shown by other workers. A restricted caloric intake decreased the number of deaths early in the life span of the rat.

2. Respiratory infections were not appreciably affected by restricted caloric intake; however, the number of instances of tumors was decreased.

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# EFFECTS IN YOUNG MEN CONSUMING RESTRICTED QUANTITIES OF B-COMPLEX VITAMINS AND PROTEIN, AND CHANGES ASSOCIATED WITH SUPPLEMENTATION<sup>1</sup>

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Short term effects of moderately restricted intakes of nutrients have been described by some (1), denied by others (2, 3). Recently we have reported absence of rapid change in physical or psychomotor performance in young men consuming for five weeks a diet containing restricted quantities of thiamine, riboflavin, nicotinic acid, the "lesser-known" B-complex factors, and a moderately low level of total protein (45 grams), 94 per cent of which was vegetable in origin (4). The present report concerns the physiologic effects observed when the same restricted nutrient intake was continued for a total of 15 to 18 weeks and the rate of their disappearance or change when supplementation with individual nutrients was made.

The original plan of the experiment was primarily aimed at obtaining further information on the effect of consuming a diet containing less than normal amounts of the following lesser-known B-complex vitamins: folic acid, pyridoxine, pantothenic acid and biotin. When detailed plans were drawn up, it became apparent that no diet likely to be encountered either in civilian or military circumstances would be restricted in its content of those nutrients, without also being low at the same time in thiamine, riboflavin, niacin and animal protein. Since practical as well as purely scientific information was

<sup>1</sup> This investigation was carried out under a contract between the Office of the Surgeon General, U. S. Army, and Passavant Memorial Hospital, Chicago, Ill.; it was also supported in part by grants from the Clara A. Abbott Fund of Northwestern University Medical School, and from the Nutrition Foundation, Inc.

<sup>2</sup> The authors gratefully acknowledge the co-operation of the Brethren Service Committee, Elgin, Ill., in the selection of volunteers from Civilian Public Service Camps maintained by the Committee.

desired, the restrictions were permitted to remain in all of the categories mentioned, with a further view to determining the rehabilitative value of single or several nutrients when added in crystalline form.

The objective of the work undertaken may be stated as follows: to study the rate of change in physical and psychomotor performance and other changes associated with restricted intakes of thiamine, riboflavin, nicotinic acid, and the lesser-known B-complex vitamins, while the intake of animal protein was negligible, and that of corn approximated 27 per cent of the total calories of the diet; to study rates of recovery; and to investigate the rôle of various nutrients, singly or in groups, in causing or mediating the recovery.

The plan of the experiment, it is to be emphasized, contemplated no starvation. Rather the plan was to provide always an adequate caloric intake as judged by maintenance of original body weight. We were unable to achieve this continuously throughout the experiment primarily because of monotony of diet and reduced appetite. This in turn caused moderate to slight loss of weight in some of the subjects, and perhaps should be included in the interpretation of the results.

**PROGRAM. General.** The subjects were seven Civilian Public Service volunteers, aged 23 to 28, banded together under the sponsorship of the Brethren Service Committee, Elgin, Illinois. The men had volunteered for this type of scientific investigation because of a humanitarian motivation which we found to be a prime factor in assuring that the subjects' responsibilities were always carried out without fail, even though there was no close supervision or any restriction of the men's activities. A careful running check of all biochemical and other data was kept throughout the experiment as a sound scientific principle, but it was always found that the few discrepancies occurring were traceable to techniques and assays rather than to any deviation from the dietary regime on the part of the subjects. A further indication of the spirit with which this contribution was made is found in the fact that all of the men chose to continue in the experiment for appreciable periods of time after release by Selective Service. While it is usually customary in scientific writing to express appreciation in a separate section reserved for the purpose, it is desired to draw attention to it specifically at the beginning of this report, because of its importance as a primary premise in validating the results obtained.

An initial 12 weeks were spent in the process of adaptation by the subjects to the various tests that were to be used. At the end of that time it was felt that a training plateau had been reached in most categories. Our observations on rates of training during this first period using the bicycle ergometer, treadmill and step test, and on various biochemical aspects were reported separately (5, 6, 7) and are referred to here as additional background giving considerably more detail in certain aspects of the experimental procedure and results.

Throughout the 12 months' duration of the experiment, the subjects carried on their usual occupations of attending classes in the university, doing laboratory or clerical work and participating in sports, e.g., basketball, tennis, hikes, etc., according to their personal desires. Meals were eaten at regular hours, and

were prepared under the supervision of the Medical Dietetics Division, Medical Nutrition Laboratory.<sup>3</sup>

All tests, collection of samples, and other procedures were held to a rigid routine in order to exclude influence from aberrant factors. Thus, as an example, the treadmill test was always performed at the same time of day on a given day of the week, in the same sequence of men. With one exception the observers who were trained for each test continued to carry out that test throughout the experiment. When military requirements prevented this, proper continuity was assured by having the new observer trained for several weeks by the one departing. By application of these principles to all phases of the experiment, consistent results were promoted.

Urine was collected each week over the same four-day period, placed in amber-colored, one-gallon containers and kept under refrigeration at all times. At the end of each day the total volume and one-tenth aliquot were measured, the latter being transferred to a sample bottle kept in a deep-freeze unit. For the same four-day period, feces were collected in a tared one-quart jar kept in the deep-freeze unit at all times. At the end of each period the samples of both urine and feces were thawed and, after homogenization by hand stirring, were analyzed promptly. In some cases, fecal collection was continued slightly beyond the four-day period to allow the collection of at least three complete stools. Routine blood chemistry was done weekly. An extra identical meal was prepared one day each week and analyzed promptly.

*Diet and supplementation.* The following periods were used:

*Control subjects (C-1 and C-2)*

Control weeks 1-12: Normal diet

Experimental weeks 1-5: Experimental diet

Experimental weeks 6-36: Experimental diet plus full supplementation

Experimental weeks 37-39: Luxurious diet in quantities desired individually

Total weeks: 51

*Experimental subjects (E-3, E-4, E-5, E-6 and E-7)*

Control weeks 1-12: Normal diet

Experimental weeks 1-15 (E-3, E-4, E-5); 1-18 (E-6 and E-7): Experimental diet

Experimental weeks 16-36 (E-3, E-4, E-5); 19-36 (E-6 and E-7): Experimental diet plus additive supplementation with crystalline B-vitamins and protein

Experimental weeks 37-39: Luxurious diet in quantities desired individually

Total weeks: 51

The normal diet fed for the first 12 weeks approached in nutrient content the recommended daily allowance levels of the National Research Council. The experimental diet was fed thereafter and contained foods frequently consumed in certain parts of this country (table 1). The latter diet was similar to the normal diet in caloric and fat content but lower in protein by some 20 grams, which were replaced by carbohydrate.

The vitamin (except ascorbic acid and pro-vitamin A) and mineral content of

<sup>3</sup> Subjects were fed at Passavant Memorial Hospital, Chicago, Illinois.

the experimental diet were markedly lower than in the normal diet, as shown by chemical, fluorometric and microbiologic assay. Two subjects were chosen as "controls"<sup>4</sup> and were given animal protein (calcium caseinate) incorporated directly into their food, and crystalline vitamin supplements in the form of capsules and tablets containing the quantities of nutrients necessary to equal or exceed slightly the amounts found in the normal diet. Placebos identical in appearance were given to the experimental subjects. Table 2 shows in summary the levels of nutrients provided for both the experimental and the control subjects. In addition, daily supplementation with pharmacal preparations of calcium and iron increased the mineral intake of all subjects to recommended levels. Water was consumed *ad libitum* throughout.

The actual intake of nutrients remained as shown in table 2 until about the 10th to 13th experimental week. At that point, anorexia, probably due to the monotony of the diet, caused refusals of varying amounts of food. In order to remove this obstacle to the objectives of the study, two additional menus having

TABLE 1  
*Sample menu of experimental diet*

<i>Breakfast</i>	<i>Dinner</i>
Applesauce	Baked hominy grits
Fried cornmeal mush	Hot beets
Fried salt pork	Perfection salad
Karo syrup with maple	Plain cornmeal muffins
Oleomargarine	Oleomargarine
	Cranberry sauce
	Apple betty
<i>Lunch</i>	<i>Bedtime</i>
Baked spaghetti casserole with corn muffin crumb topping	Cornmeal muffins
Green beans	Grape jelly
Carrots	Lemonade
Pickle relish	Sugar
Plain cornmeal muffins	
Oleomargarine	
Pear halves	

similar nutritional content as that of the single diet shown in table 2 were introduced at week 23. Refusals of food decreased markedly thereafter.

At the beginning of the 16th week of the experimental diet, supplementation with crystalline nutrients was begun for three of the experimental subjects. The remaining two subjects were supplemented 3½ weeks later—the 19th week (see schedule below). The general plan of supplementation was to provide first the "better-known" B-complex factors or protein, ending finally with the "lesser-known" factors. The actual schedule is presented in the following summarized form:

Experimental week 1: Experimental diet begun for all 7 subjects and continued for 5 weeks.

<sup>4</sup> Assuming the crystalline supplements to be nutritionally complete.

Experimental week 6: Experimental diet continued for subjects E-3, E-4, E-5, E-6 and E-7; and experimental diet plus supplements<sup>5</sup> for control subjects C-1 and C-2.

Experimental week 16: 1.2 mgm. thiamine added to diet of subjects E-3, E-4 and E-5 (total daily intake then became 1.75 mgm.).

Experimental week 19: 25 mgm. thiamine given intravenously to all 7 subjects and thiamine added to the diet of E-6 and E-7 as it had been 3 weeks earlier to E-3, E-4 and E-5. (From this time on, therefore, all subjects were receiving added crystalline thiamine.)

Experimental week 21: 25 mgm. thiamine given intravenously to all 7 subjects, and subject E-3 was given in addition 10 mgm. riboflavin and 200 mgm. nicotinamide per day in addition to the amounts of these nutrients contained in the diet (total intakes then 1.75 mgm., 1.85 mgm., and 18.8 mgm., respectively). Subjects E-3, E-4 and E-5 received 45 grams of calcium caseinate in their food starting this date.

TABLE 2  
*Nutritional content of the normal and experimental diets*

NUTRIENTS		NORMAL DIET	EXPERIMENTAL DIET
Calories.....		3170*	3300*
Protein.....	gram	70*	45
(l-Tryptophane).....	mgm.	700-900	210-300
Calcium.....	gram	0.86*	0.20*
Phosphorus.....	gram	1.26*	0.58*
Iron.....	mgm.	15.5*	12.0*
Thiamine.....	mgm.	1.44	0.50
Riboflavin.....	mgm.	1.84	0.30
Niacin.....	mgm.	15.6	5.8
Biotin.....	mcg.	44	19
L. casei factor.....	mcg.	64	23
Pantothenic acid.....	mgm.	4.7	1.1
Pyridoxine.....	mgm.	1.7	1.1
Ascorbic acid.....	mgm.	105*	90*
Vitamin A.....	I. U.	7400*	16600*

\* Calculated.

Note: Normal diet consumed by all subjects for first 12 weeks; thereafter all subjects, both control and experimental, consumed experimental diet but former received supplements in quantities to equal or slightly exceed levels of nutrients found in normal diet (see footnote 5).

Calcium, phosphorus and iron levels of the experimental diet were increased to 0.90 gram, 1.1 grams and 32 mgm., respectively by use of dicalcium phosphate and iron pyrophosphate.

Ascorbic acid content of experimental diet was provided in part by synthetic lemon powder fortified with ascorbic acid.

Vitamin A content of experimental diet was mostly in the form of betacarotene.

<sup>5</sup> The supplements were as follows: (1) 40 grams animal protein (as 45 grams calcium caseinate); (2) 0.70 gram calcium as dicalcium phosphate; (3) 0.54 gram phosphorus as dicalcium phosphate; (4) 20 mgm. iron as iron pyrophosphate; (5) 666 I.U. vitamin D; (6) 1.2 mgm. thiamine hydrochloride; (7) 1.5 mgm. riboflavin; (8) 12 mgm. nicotinamide; (9) 60 mcg. biotin; (10) 90 mcg. L. casei factor (folic acid); (11) 6 mgm. pantothenic acid, half as racemic calcium pantothenate and half as dextro calcium pantothenate; (12) 300 mcg. para-aminobenzoic acid; (13) 3 mgm. pyridoxine hydrochloride; (14) 0.50 gram choline chloride as choline dihydrogen citrate.

- Experimental week 22: Subject E-3 began receiving 6 lesser-known B-complex factors in quantities shown in footnote 3. (From this date, therefore, subject E-3 received all nutrients supplied to the control subjects.)
- Experimental week 23: Two new menus were added to make a three-day cycle in place of the same diet that had been fed each day up to this point. The average nutrient content of these menus was unchanged from that fed previously.
- Experimental week 27: All 7 subjects received orally 100 mgm. nicotinamide at each of 3 meals, and for the following 5 days received 50 mgm. nicotinamide at each meal—12 mgm. per day were given thereafter. At this point also, protein was added (as calcium caseinate) to the diet of subjects E-6 and E-7. Subjects E-3 and E-4 received the following food items added to their diet: (a) 1 egg for breakfast; (b) 100 grams (raw weight) ground beef for lunch; (c) 1 half pint whole milk for dinner. In order to keep the caloric and protein content similar to that of the other subjects, the calcium caseinate, sugar and oleomargarine were appropriately reduced.
- Experimental week 28: All 7 subjects received 5 mgm. riboflavin orally at each meal for 7 days and 1.5 mgm. per day thereafter. (At this point, all subjects were therefore getting supplementary protein, thiamine, riboflavin and nicotinamide; subjects E-3 and E-4 received egg, meat and milk; subjects E-4, E-5, E-6 and E-7 lacked the lesser-known B-complex supplement.)
- Experimental week 31: Subject E-4 received 6 lesser-known B-vitamins (see footnote 5).
- Experimental weeks 33 to 36: Beginning at different times during this period subjects C-1, E-3, E-5, E-6 and E-7 received daily, 4 grams sulfathalidine—1 gram at each meal and 1 gram before retiring.
- Experimental weeks 37 to 39: All subjects ate a luxurious diet *ad libitum* and received an additional supplement of specially grown primary brewer's yeast (Abbott). The total daily intake of nutrients provided by the two supplements alone (i.e., exclusive of diet) was as follows: 2.4 mgm. thiamine, 3.5 mgm. riboflavin, 12.0 mgm. niacin, 85.0 mcg. biotin, 7.5 mgm. pantothenic acid, 3.4 mgm. pyridoxine, 0.43 mgm. p-aminobenzoic acid, 540.0 mgm. choline, 40.0 mgm. inositol, and 90.0 mcg. L. casei factor (from tablets only, L. casei factor not analyzed in the yeast).

PROCEDURES. I. *Tests of Physical Performance.* 1. *Electrodynamic brake bicycle ergometer.* A stationary bicycle frame arranged for a variable, electrically controlled resistance against rotation of the pedals. A double work period to exhaustion of the leg muscles was used, separated by a 10-minute rest on a cot. Measurements were made in terms of duration of each ride, and resting and post-exercise pulse rates.

2. *Treadmill.* A standard run with a belt speed of six m.p.h. up a 10 per cent grade. Two runs of four minutes each were used with an intervening 10-minute rest. Measurements were made in terms of post-exercise pulse rate. Data were also obtained on resting pulse rates.

3. *Harvard step test.* A 20-inch step test with 30 up-down cycles completed per minute for five minutes. Measurements were made of resting and post-exercise pulse rates and blood pressures.

A cardiometer was used in connection with these three tests during the latter part of the experiment for continuous recordings of the heart rate. A record was taken before exercise, during the entire exercise, and for several minutes after exercise.

II. *Tests of Psychomotor or Psychologic Response.* 1. *Johnson code test.* A

mentation measurement using code letter substitutions. The time required to complete a given number of problems was recorded, as was the number of errors.

2. *Hand steadiness test.* A device for measuring number and duration of contacts made between a perforated plate and a stylus held in subject's outstretched hand, contacts being recorded for movements in all directions.

3. *Single dimension pursuit test.* An instrument developed by the School of Aviation Medicine, Randolph Field, Texas, which records the total time an artificial, moving horizon is kept in line with the reference points of the instrument.

4. *Rotary pursuit maze.* A maze pattern in a rotating metal drum which is traced with a stylus by the subject, arranged to record number and duration of contacts for a standard period of time.

5. *Pursuit meter.* A miniature airplane device which records errors in correcting the positions of the indicator in three planes, involving separate use of both hands and the right foot.

6. *Ataxiameter.* A device for recording body sway. The ataxiameter records sway along any radius of a circle. The data are resolved to two directions. The subject's eyes are closed during the test.

7. *Minnesota multiphasic personality inventory.* A psychometric device consisting of 550 statements to be classified by the subject into three categories (true, false, or cannot say). Indications of personality deviation toward any of the following syndromes may be obtained: hypochondriasis, depression, hysteria, psychopathic personality, masculinity-femininity, paranoia, psychasthenia, schizophrenia, and hypomania (8).

III. *Biochemical.* The biochemical method for analysis of urine, blood, food and feces were as follows, with appropriate modification being made in preparation for assay according to the type of material:

1. *Thiamine.* The method of Hennessy and Cerecedo (9) as modified by Friedemann and Kmiecik (10), (fluorometric).

2. *Riboflavin.* The method of Ferrebee (11) with slight modification (fluorometric).

3. *L. casei factor (folic acid).* The method of Teply and Elvehjem (12), (microbiologic).

4. *Pantothenic acid.* The method of Hoag et al. (13), (microbiologic). Crystalline vitamin solutions were substituted for the yeast and vitab mixtures.

5. *Biotin.* Originally by the method of Shull and Peterson (14), (microbiologic). Later the method of Teply and Elvehjem for folic acid was used for biotin assays.

6. *Pyridoxine.* A method based on that of Stokes and co-workers using *Neurospora sitophila* (15), (microbiologic).

7. *Nicotinic acid.* The method of Krehl and co-workers (16), (microbiologic).

8. *N<sup>1</sup>-methylnicotinamide (F<sub>2</sub>).* A modification of Najjar's method (17), (colorimetric).

9. *1-Tryptophane.* A method based on that of Wooley and Sebrell (18), (microbiologic).

10. *Lactic acid.* The method of Friedemann and Graeser (19), (titrimetric).

11. *Pyruvic acid*. The method of Friedemann and Haugen (20), (colorimetric).

12. *Creatinine*. A method based on that of Peters (21), (colorimetric).

13. *Ascorbic acid*. The method of Roe and Kuether (22), (colorimetric).

14. *Feces and food*. Samples were prepared for folic acid, pantothenic acid and biotin assay by the procedures outlined by the University of Texas workers (23).

15. *Blood*. The following routine determinations on the blood were made: urea nitrogen, non-protein nitrogen, total serum protein, albumin-globulin ratio, icteric index, cephalin-cholesterol flocculation, alkaline phosphatase and bromsulphalein excretion.

IV. *Clinical Evaluations*. 1. *Physical examinations*. Made initially, with periodic nutritional examinations carried out thereafter.

2. *Log*. Twice weekly each member of the group reported all symptoms that had occurred or were still present, and these were recorded. Every effort was made to encourage the reporting of all items that might in any way be related to the diet or the health and well-being of the subjects.

3. *Blood*. Finger-tip blood was taken weekly and the following determinations made: erythrocyte, leucocyte and platelet enumeration; hematocrit volume, sedimentation rate, reticulocyte count, hemoglobin concentration and differential leucocyte count.

4. *Gastro-intestinal series*. X-ray photographs and fluoroscopy were made to determine the emptying time of the stomach and the degree of activity and pattern of the small intestine.

5. *Electrocardiogram and basal metabolic rate*. Determinations were made as a part of the first physical examination and periodically throughout the experiment.

6. *Ultraviolet irradiation*. A series of exposures to ultraviolet light was made on the left arm of each subject. The light was applied for 10 minutes from a distance of six feet. A General Electric Sun Lamp was used.

RESULTS. Part 1—Up to the first supplementation made at week 16 for subjects E-3, E-4 and E-5, and week 19 for subjects E-6 and E-7.

I. *Biochemistry*. The first changes found to occur were, as expected, in the biochemical aspects. A marked drop in the urinary excretion of almost all the vitamins under consideration occurred in all seven subjects within the first two weeks of the restricted nutrient intake. The average daily urinary thiamine excretion approached zero by the end of the first five weeks on the experimental diet, and the load test response (tissue saturation test) carried out at that time likewise was much lower than that found previously at the end of the normal diet period. Similar findings were obtained for riboflavin, N<sup>1</sup>-methylnicotinamide, pyridoxine and pantothenic acid. Decreases in biotin and folic acid were definite but not as marked as in the above instances: the average levels found were 10 mcg. and 2 mcg., respectively, per 24 hours. When, at the end of the five-week period, supplements were given to subjects C-1 and C-2 with the intent of making them controls, the increase in excretion levels was pronounced



for thiamine, biotin and pantothenic acid, and somewhat less for riboflavin, N<sup>1</sup>-methylnicotinamide and pyridoxine.

The average daily urinary excretion levels for the last 10 weeks prior to supplementation were: thiamine, 14 mcg.; riboflavin, 151 mcg.; nicotinic acid, 0.7 mgm.; N<sup>1</sup>-methylnicotinamide, 0.4 mgm.; biotin, 14 mcg.; L. casei factor, 2.4 mcg.; pantothenic acid, 0.8 mgm.; pyridoxine, 0.2 mgm. These values may be compared with those of the two control subjects whose urinary excretion levels at that time averaged as follows: thiamine, 320 mcg.; riboflavin, 517 mcg.; nicotinic acid, 0.9 mgm.; N<sup>1</sup>-methylnicotinamide, 3.2 mgm.; biotin, 63 mcg.; L. casei factor, 2.9 mcg.; pantothenic acid, 3.7 mgm.; pyridoxine, 0.4 mgm. Typical excretion curves are shown in figure 10.

The resting and post exercise pyruvic acid levels of the blood were found to be high in the case of several of the experimental subjects. The load test responses for thiamine, riboflavin and N<sup>1</sup>-methylnicotinamide were low in the experimental subjects. In the two control subjects, the load test responses had reached the initially determined normal levels for both individuals, and the blood pyruvic acid levels also were within normal limits.

The urinary excretion of free l-tryptophane did not drop as might have been expected in view of the low animal protein content of the diet and the relatively high corn content. The detailed findings on this aspect were reported separately (24). Another unexpected finding was the steady drop in 24-hour creatinine excretion shown by the experimental subjects. There was a marked drop in the creatinine excretion of all subjects when the experimental diet was introduced. For the experimental subjects this decrease continued, but was arrested in the controls when they received the supplement of animal protein. The latter findings are reported in a separate communication (25).

No striking change was observed in any of the cellular components of the blood. No change from original levels of total serum protein concentration was observed in any of the seven subjects. Total serum protein concentration did not lie outside the normal range at any time during the 15-week period nor subsequently. Variations were seen in the A/G ratios which were not consistent within either the experimental or the control groups.

Plasma carotene dropped markedly and uniformly in all seven subjects, while plasma vitamin A dropped only slightly in a few subjects. This is an interesting finding in view of the fact that the estimated beta-carotene intake in the experimental period was approximately double that in the normal diet period, the calculated vitamin A and beta-carotene being respectively 3000 and 4000 in the former period, and 1000 and 9000-15000 in the latter, in terms of I.U. of vitamin A potency.

Whole blood ascorbic acid showed a uniform pattern in all seven subjects, lower than original levels (but not lower than normal) being found on two occasions—experimental weeks 24 and 25.

One of the most consistent biochemical findings concerned the vitamin content of the feces. In contrast to the urinary levels which decreased promptly as described above, the levels in the feces did not decrease at any time during the first 15 experimental weeks from those found during the normal diet period; all

fecal vitamin levels except nicotinamide and pyridoxine exceeded intakes. Supplementation with crystalline vitamins had no apparent effect upon these levels in the feces (fig. 10). The detailed results pertaining to urinary and fecal vitamin excretion levels were presented in separate reports (6, 7, 34).

II. *Physical Performance*. Short term effects—i.e., at the end of five to six weeks of the restricted intake—appeared to be negative. However, retrospect examination of certain of the individual scores indicate the possibility that in at least two instances, subjects E-3 and E-4, slight changes were present which later became statistically significant. These short term effects were discussed in some detail in a separate report (4).

Before the end of week 15 or week 18<sup>6</sup> of the experimental period, there had been a definite decrease in the ability of the experimental subjects to do the physical work which had been within each subject's capability during the normal diet period. This decrease was reflected in three ways: (a) elevated post-exercise pulse rates, (b) inability to complete the step and treadmill tests, and (c) inability to ride on the bicycle ergometer for as long a period as originally. These findings are presented graphically in figures 1, 2 and 3. Subjects E-4, E-5, E-6 and E-7 now were able to run an average of about three minutes for the first test on the treadmill and somewhat less for the second. It will be recalled that four minutes was the time prescribed to finish each test. There was no question in our minds about the actual impairment in ability of the subjects to finish these physical tests, since the post-exercise pulse rates were as high or higher than those when the tests had been successfully completed. As a further test of the matter, the grade of the treadmill was lowered without the prior knowledge of the subjects, and they then completed the test. This was done on three occasions. When the original grade was restored, the inability of the subjects to finish the test immediately re-appeared except for subject E-4 who had received thiamine meanwhile.

Table 3 indicates changes which occurred by the time the first supplement, thiamine, was given. It is important to note the general lack of deterioration and the occasional occurrence of some improvement in performance of the control subjects. The general decrease in performance<sup>7</sup> of the experimental subjects can be contrasted with these results. The greatest over-all change occurred in the ergometer and step test. However, when the improvement on the treadmill by the control subjects is considered, it is difficult to escape the conclusion that lowered performance was found in all three physical tests. This conclusion is borne out in the minds of the observers who recall quite readily the relative ease with which the subjects had originally gone through these tests, and the easy fatiguability that developed during the experimental diet period. In the later part of this period the difference between the two groups—i.e., control and experimental—was notable.

One of the interesting developments observed in connection with the measurement of physical performance was a definite and, in some instances, a marked

<sup>6</sup> Depending upon time of first supplementation.

<sup>7</sup> Step test performance did not, however, fall below those that we have usually found in the random cross section of infantry soldiers doing the test for the first time.

rise in resting pulse rate in four of the experimental subjects.<sup>8</sup> At the end of 15 weeks this rise amounted to some 20 beats per minute for subject E-3 but somewhat less for the others. No similar rise was found in the controls (fig. 7). A rise in resting diastolic blood pressure was also found to occur in three of the experimental subjects (fig. 8). This elevation in resting pulse rate and diastolic pressure, and the effects associated with supplementation will be discussed later in this report.

III. *Psychomotor and Psychologic Response.* The uniformity of lowered performance found in the physical efficiency category was not so evident in the psychomotor tests. Thus, in table 3, it is seen that while certain of the experimental subjects showed poorer scores for some tests, some of the same group also showed better or unchanged scores. Rather conclusive evidence of an

TABLE 3  
Change\* in performance of tests at end of 15-18 weeks†

SUBJECT	PHYSICAL EFFICIENCY			PSYCHOMOTOR RESPONSE								CHANGE IN BODY WEIGHT
	Treadmill (post-exercise pulse rate & completion of exercise)	Bicycle ergometer (dura- tion of effort)	Step test (post-exercise pulse rate & completion of test)	Single dimension pursuit test (steady time on center)	Pursuit meter (number of errors)	Johnson code test (time required to complete test)	Rotary maze		Hand steadiness		Ataxiometer (steadiness in standing as measured by swaying)	
							Number of errors	Duration of errors	Number of errors	Duration of errors		
C-1†.....	+2	-1	0	+2	+3	+1	+1-+2	0	0	0	0	0
C-2.....	+2	0-1	+1	0	-1	0	0	-2	0-1	-1	0	0-1
E-3†(15 weeks).	-1	-3	-3	+1	0	0	+1	0	0	-1	0-1	-1-2
E-4 (15 weeks).	-2	-3	-3	-1	0	0	0-+1	0	+2	0	-2	-2
E-5 (15 weeks).	-?	-2	-?	-1	0	0	+2	+1	+3	+2	0	-1-2
E-6 (18 weeks).	-1	-2	-3	-1	+1	-1	-1-2	0	0	0	-1	0
E-7 (18 weeks).	-2	-2	-2	0-1	-1-2	0	0-1	0-1	-0	-1	-2	-1-2

\* Scored as follows: 0 = approximately no change from performance on normal diet. Minus or plus 1 = slight deterioration or improvement, respectively. Minus or plus 2 = moderate deterioration or improvement, respectively. Minus or plus 3 = definite deterioration or outstanding improvement, respectively.

† Refers to number of weeks on restricted nutrient intake before first supplementation (thiamine) was given.

‡ C = Control, E = Experimental.

—? Evidence indicates decrease in performance but record for this subject incomplete due to ankle injury sustained while playing basketball.

increased tendency to sway was found to occur in the experimental group by the 16th to 18th week as measured by the ataxiometer. There was a marked increase in sway in at least three of the five subjects, and possibly in four, while no change was observed in the two control subjects. Ataxiometer, single pursuit meter and rotary maze data are presented graphically in figures 4, 5 and 6.

<sup>8</sup>The 5th subject had an incomplete record due to an injury sustained while playing basketball.

Further discussion of our interpretation of the changes in the psychomotor and psychologic responses will be withheld until the effects of supplementation are presented later in this report.

IV. *Clinical Findings.* As mentioned earlier, there was a decrease in food intake caused by the monotony of a single menu and/or possibly by a decrease in

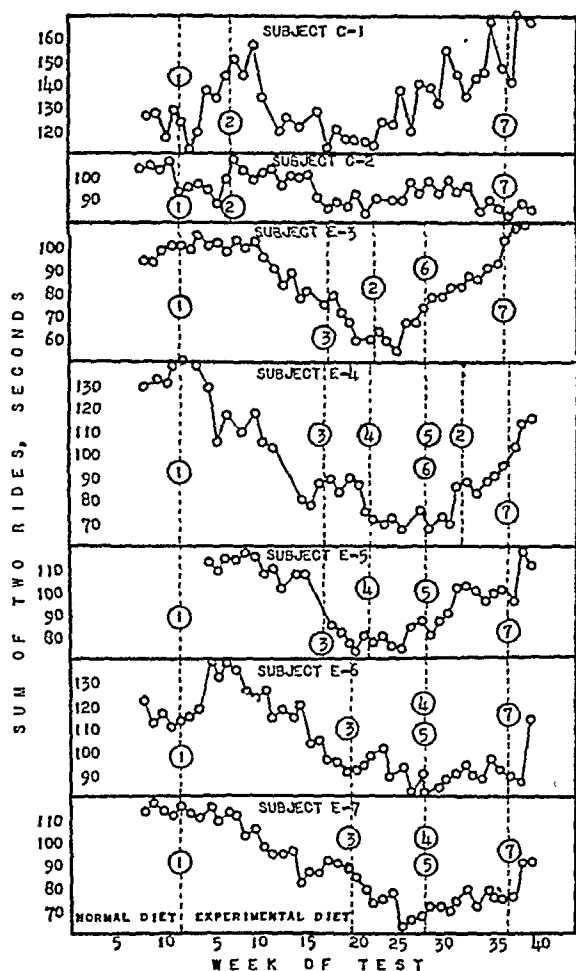


Fig. 1

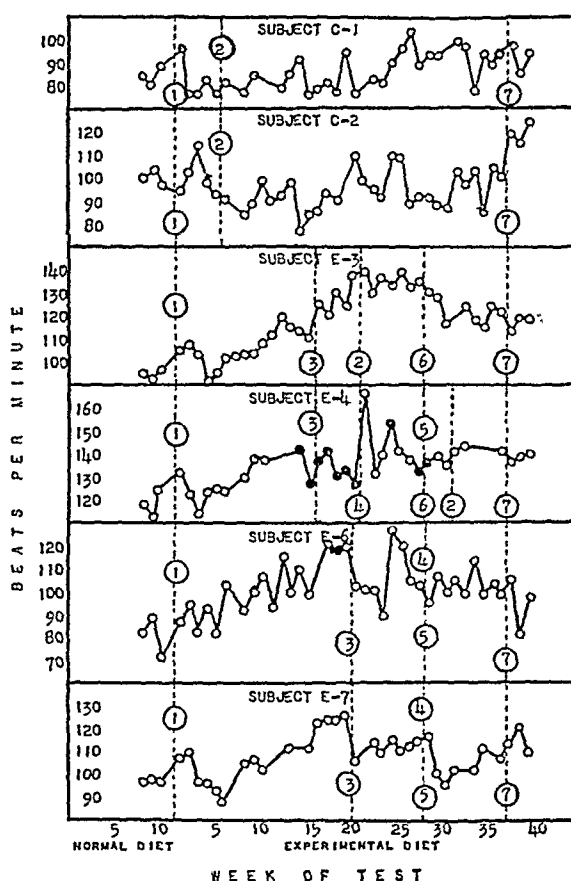


Fig. 2

Fig. 1. Duration of ergometer effort.

Fig. 2. Pulse rates following step test. Pulse rates are those taken during the interval 1-1½ minutes post-exercise. Tests during which subjects were unable to continue for the five minutes prescribed are designated by filled circles.

appetite. The calories provided by the experimental diet were approximately equal to those of the normal diet, while the amount of unscheduled activity was left to the discretion of the individual. Beginning with the 8th week of the experimental diet period, some food was refused. This continued until the 23rd week, when two additional menus were introduced. A loss in body weight ranging from 6 to 11 lbs. in four of the experimental subjects was caused by this decreased caloric consumption.

One of the early problems arising during experimental weeks 5 to 13 was the occurrence of localized superficial mouth ulcers. These were referred to by the

subjects as "canker sores" and apparently occurred more frequently in the experimental than in the control subjects. The resemblance between these lesions and similar ones reportedly associated with a deficiency of adenylic acid was considered (26, 27, 28). They persisted for approximately ten weeks and remitted spontaneously before the introduction of supplements. There was no associated herpes simplex or Vincent's infection.

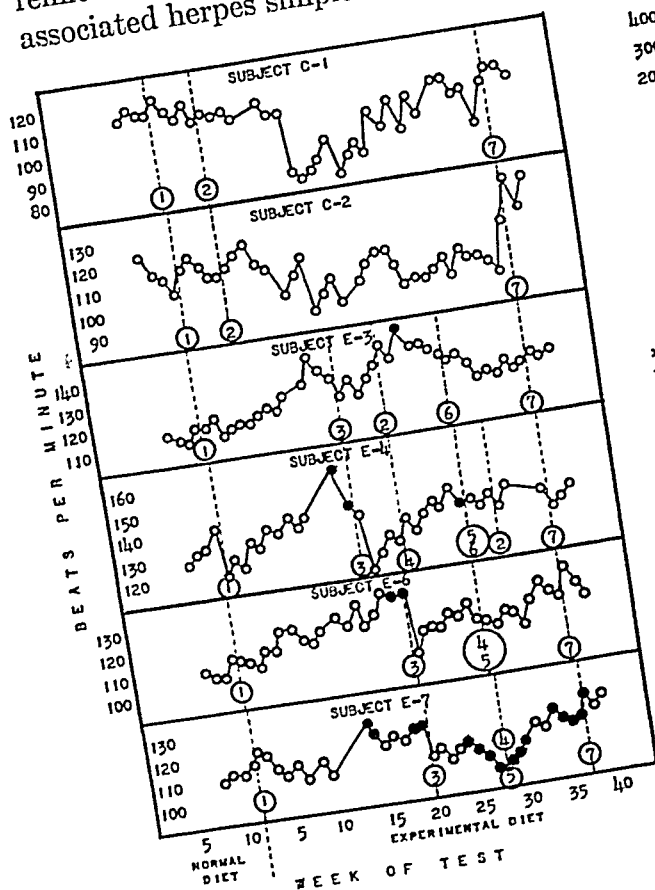


Fig. 3

Fig. 3. Pulse rates following treadmill run. Pulse rates are those taken during the interval 1-1½ minutes post-exercise. Tests during which subjects were unable to continue for the four minutes prescribed are designated by filled circles. The grade of the treadmill was reduced from 10 per cent to 8.6 per cent on experimental weeks 15, 16 and 17.

Fig. 4. Ataxiameter test.

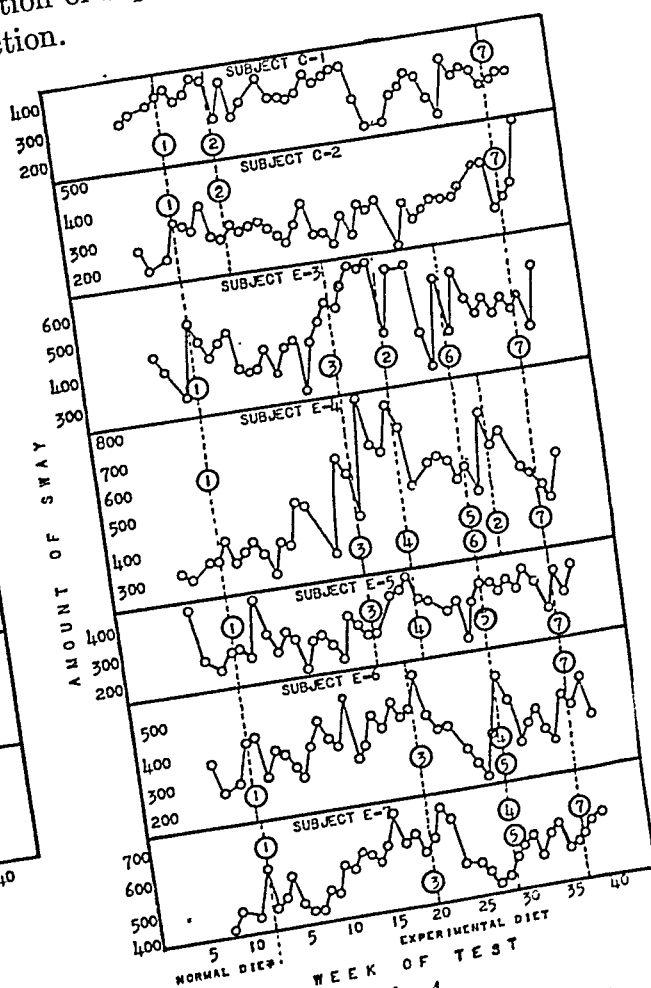


Fig. 4

Following this, several of the experimental subjects complained of sore mouths and this posed a real difficulty because of the irritation by the cornmeal in the diet. Some increase in size and number of red-centered papillae was observed on the lingual tips of the experimental subjects. As the experimental period progressed, lingual edema, evidenced by the presence of tooth imprints along the margin of the tongue, was observed. In addition, subject E-7 demonstrated a fiery erythema, first appearing on the under surface of the tongue and later spreading to involve the buccal mucosa as well. This sign fluctuated in

degree but persisted for approximately four weeks and then spontaneously remitted. The soreness associated with these lesions was the most pronounced when the subjects consumed hot or acid foods and liquids.

Occasional oral stigmata sometimes associated with riboflavin deficiency were observed. Subject E-3 demonstrated a typical magenta tongue on several occasions during this period although the finding was not constantly present. This subject, and one other, presented bilateral angular stomatitis and cheilosis

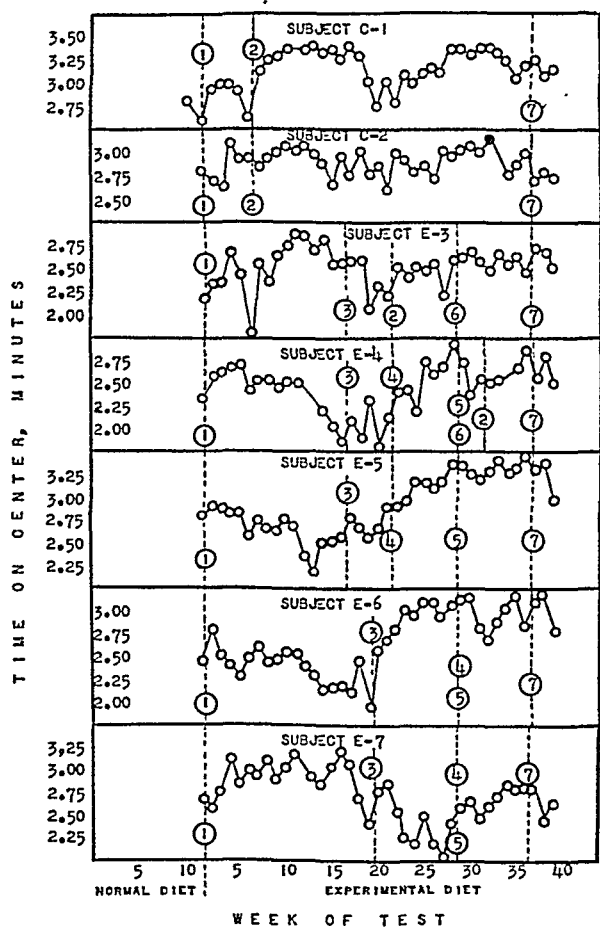


Fig. 5. Single pursuit test.

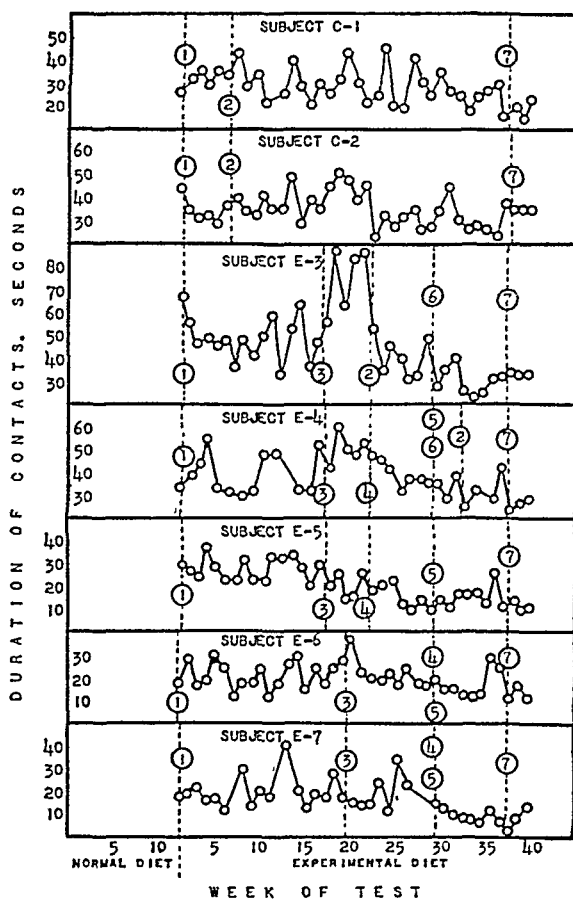


Fig. 6. Rotary maze test.

immediately following some dental work. The angular stomatitis in the subject with the magenta tongue persisted for six to eight weeks. Interpretation of this finding was complicated somewhat, since two other subjects (one a control) developed unilateral angular stomatitis also while undergoing dental treatment. The lesion in the control subject healed promptly, however.

At approximately the 12th week of the experimental period an erythematous lesion of the scrotum appeared in one of the experimental subjects and was subsequently noted in two others. Starting out as an area approximately three centimeters in diameter, the lesions spread to involve about half of the anterior scrotal surface. There was associated burning, thickening, pigmentation, and dry desquamation. During the same time, all experimental subjects frequently reported burning and pain of the anus and perianal region, this being more

severe during and immediately after defecation. Examination of the region revealed increased moisture and erythema. Because of the controversy, which has long existed, over the rôle of light in the production of pellagrous dermatitis (29, 30, 31), the left arms of all subjects were exposed daily to a sunlamp for a period of four weeks. The intensity of the irradiation was kept at a level just below that needed to produce erythema. The failure of characteristic skin lesions to appear on the arm and hand during this irradiation may be interpreted as: (a) insufficient degree of

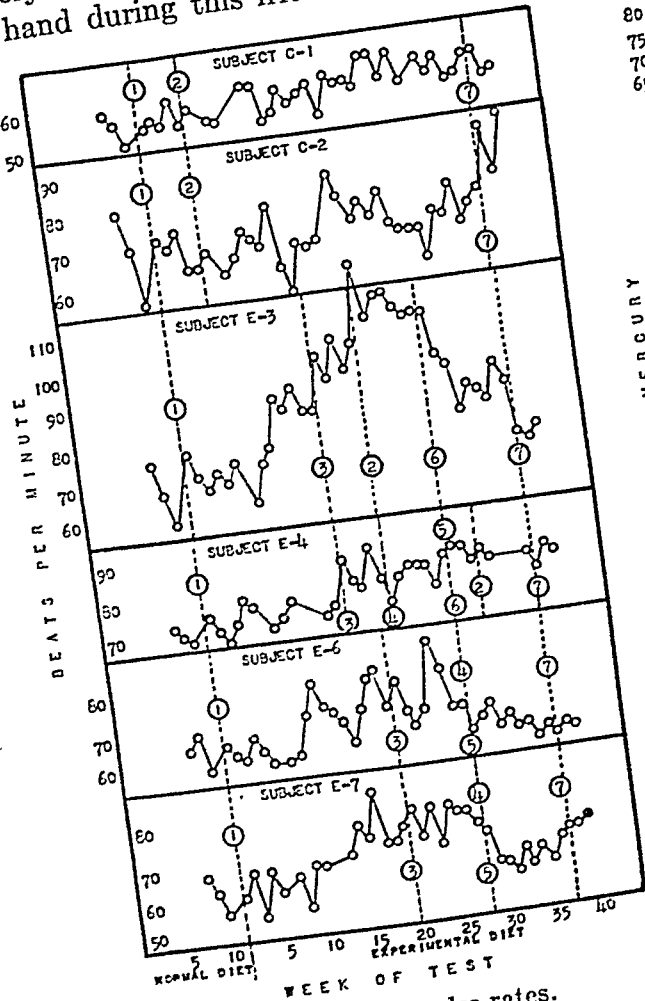


Fig. 7. Resting pulse rates.

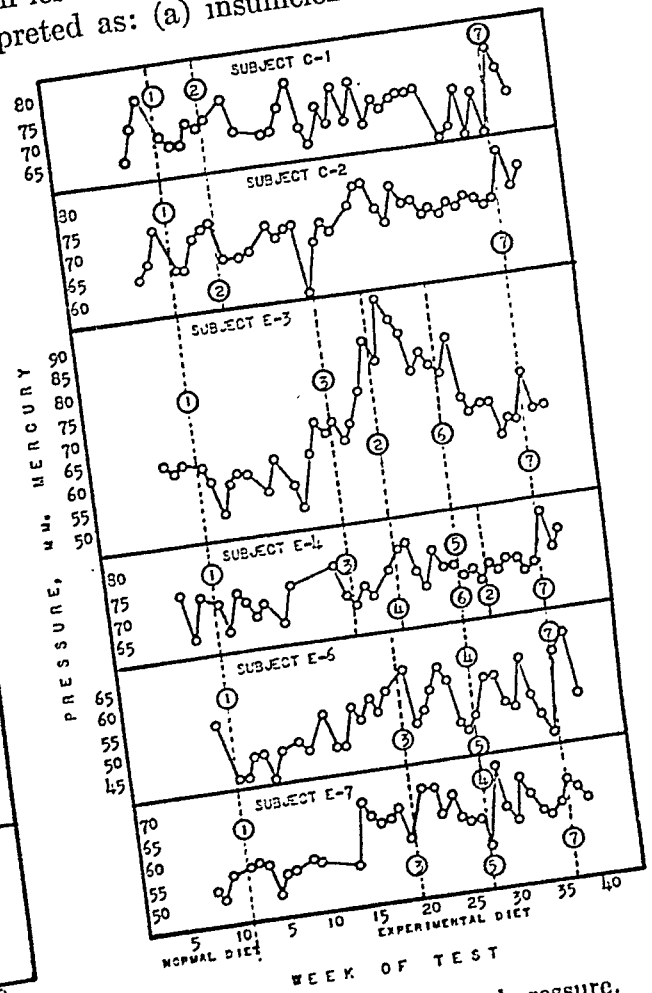


Fig. 8. Resting diastolic blood pressure.

dietary deficiency; (b) insufficient time and intensity of exposure, or (c) lack of association between sunlight and the production of pellagrous dermatitis. One control and one experimental subject, C-1 and E-7, demonstrated occasional cardiac arrhythmia after exercise on the treadmill and step test. Subject C-1 presented a slow, irregular rhythm beginning during the first minute post-exercise and lasting about 60 seconds. The rate 120-150 seconds post-exercise during these episodes was greater than the 60-90 seconds post-exercise. This was observed infrequently and was unassociated with any obvious cause. He was found to have resting bradycardia as well (44-50 beats per min.). Subject E-7 showed typical coupled rhythm, usually consisting of a normal beat

followed by a ventricular premature contraction. Variation in the shape of the ventricular complex indicated a shifting focus of excitation. This arrhythmia began one minute post-exercise and lasted approximately two minutes. We were informed that during previous experimental work this finding had been observed also following a test on the bicycle ergometer. It is also possible that it had occurred on other occasions, particularly following strenuous exercise, such as basketball, when no measurements were made. The first instance during this experiment occurred about six weeks after the change to the experimental diet. The frequency of occurrence increased, and subsequently gradually decreased without apparent association with supplementation by any one nutrient. Resting electrocardiograms showed no aberration except a prominent "U" wave.

Numerous subjective symptoms were reported with sufficient constancy to merit mention. Included in these were itchiness of the face, feelings of "spider webs" on exposed portions of the body, lassitude, inability to remain soundly asleep at night and excessive tiredness. Some of these general symptoms were so apparent in the experimental subjects that the differentiation between the two groups was obvious to the trained observer and even to the subjects themselves. There were gastro-intestinal symptoms described as heartburn and a feeling of fullness after eating, with some flatulence. Anorexia which was relieved by a greater variety in the menu, some vomiting, and some post-exercise nausea were reported upon occasion.

After about 15 weeks of restricted intake, aching of the feet was reported by one subject and this subsequently appeared in all experimental subjects, although to a minor extent in three of them. It did not occur while walking, but at night it was frequently severe enough to interfere with the sleep of the two subjects most affected. The aching could be relieved by elevation of the feet and by exercise. Subsequently burning of the feet was also reported by these two subjects, and this was described as being similar to the feeling following frostbite. In general, the symptoms were similar, but in less degree, to those described recently by Simpson (32) and also by Harrison (33) as occurring in some prisoners of war deficient in B-complex vitamins.

Roentgenologic examination<sup>9</sup> of the gastro-intestinal tract was performed on all subjects during the control period, after 15 weeks of the experimental period, and finally during the later states when crystalline supplementation had been received. The examination made at experimental week 15 disclosed in the experimental subjects a delayed gastric emptying time, and puddling of the barium meal in the small intestine, with coarsening of the mucosal pattern. The findings, however, were not as marked as are seen in individuals who have had a deficiency state over a much longer period of time. Nevertheless, without a previous knowledge of which subjects were controls, it was possible for the roentgenologist, by means of these studies, to determine which of the individuals had been on the experimental dietary regime.

RESULTS. Part II—From beginning of supplementation at experimental

<sup>9</sup> These studies were carried out by the Department of Roentgenology, Northwestern Medical School.



week 16 or 19 through end of additive supplementation at week 35 and through three weeks of luxurious diet ending at week 39.

Changes occurred in the biochemical picture as expected in accordance with the supplementation program (fig. 10). Fasting urinary values increased promptly, and blood levels, particularly pyruvic acid following thiamine supplementation, returned to normal. The vitamin content of the feces during the period of supplementation is described in detail in a separate report (34).

I. *Supplementation with Thiamine.* One and two-tenths milligrams of thiamine per day were added to the dietary intake at experimental week 16 for three of the experimental subjects, and at week 19 for the remaining two. At week 19, 25 mgm. thiamine were given intravenously to all subjects, experimental as well as control.

a. *Physical performance.* The effect of the thiamine supplementation on most of the tests of physical performance by the experimental subjects was slight, and proved to be very temporary. On the bicycle ergometer there was some arrest of the decrease in duration of effort, and in two subjects a very slight improvement was noted. The improvement, however, was quite transient, and in all instances it became apparent within two weeks of the introduction of thiamine that no constancy in improvement had been attained (fig. 1).

In the step test, even the very temporary effect noted for the ergometer was not found in the majority of the subjects, although a rather striking exception was noted in subject E-6 (fig. 2). Previously he had shown an elevated post-exercise pulse rate, and on the last preceding occasion had failed to finish the step test. Immediately after receiving intravenous thiamine, although the subject did not know what it was, he was capable of completing the step test, and with a much lower post-exercise pulse rate. Subsequently the post-exercise pulse rate again rose and then dropped back to a level that was still moderately higher than was observed for this subject during the normal diet period (fig. 2). The converse of this situation was seen in subject E-3, who felt no benefit from either the intravenous dose or the daily supplementation, and continued to have a fast post-exercise pulse rate for five weeks following the first addition of thiamine to the diet. No improvement was observed in subject E-4 and only slight improvement in E-7.

No effect was apparent on systolic or diastolic blood pressures. No effect had been observed on the former as the restriction progressed, but in the case of the latter, it will be recalled, there had been a definite rise in the post-exercise reading for two of the subjects. In addition, the resting diastolic pressure had risen in three experimental subjects.

Performance on the treadmill showed some improvement insofar as post-exercise pulse rates were concerned. However, this improvement was also transient, with the previously high levels recurring within a few weeks (fig. 3). Subject E-7, who had been unable to complete the test before thiamine supplementation, again showed this inability. Only subject E-6 seemed to show improvement which was not transitory.

In brief, following supplementation with thiamine alone, there was a fleeting

improvement in the amount of work that could be done, and in post-exercise pulse rates, but the *temporary* nature of this improvement was a much more striking phenomenon.

*b. Psychomotor and psychologic response.* The change in psychomotor scores followed the pattern described above; one subject appeared to benefit markedly, whereas another, on exactly the same regime, continued to perform at lowered score levels. Thus subject E-6 presented improved scores on the rotary maze (decreasing both the duration and the number of errors), and also on the pursuit meters; conversely, subject E-3 showed either no change on those tests or performed even more poorly (figs. 5 and 6). In general, the same picture emerges in the psychomotor category as did in that of physical performance—either a temporary improvement occurred only to be followed by a regression, or no improvement was noticeable.

The amount of body sway as measured by the ataxiameter had definitely increased in four of the five experimental subjects (table 3 and fig. 4). The addition of thiamine was associated with a decrease in this tendency in subjects E-6 and E-7, the reverse being true in subjects E-3, E-4 and E-5. The improvement in ataxiameter scores noted for subjects E-6 and E-7 eventually regressed, again demonstrating temporary improvement on the part of the subjects.

II. *Supplementation with Protein (animal) and/or New Menus.* After experimental week 20, 40 grams of animal protein daily (as 45 grams calcium caseinate) were added to the diet of subjects E-3, E-4 and E-5. Subject E-3 received orally, in addition to protein, 1.5 mgm. riboflavin and 12 mgm. nicotinamide daily as well as 25 mgm. thiamine, 200 mgm. nicotinamide, and 10 mgm. riboflavin intravenously. He also received the lesser-known B-complex factors one week later. A comparison of two different degrees of supplementation is made: (a) animal protein superimposed on the thiamine which was started some five weeks earlier, and (b) full supplementation.

By this time monotony of the diet had become a factor. Accordingly, two new menus were devised, having such nutritive content that the average nutrient intake for the three-day cycle would be almost identical with that provided by the single menu. The two new menus were added at experimental week 23, and produced definite improvement in morale. The most immediate effect in terms of nutrition was to cause an increase in caloric intake. Thereafter, the refusal of food became less of a problem. It is not to be assumed that lowered caloric consumption played a major rôle in the findings, since the daily caloric intake for any one subject did not fall below an average of 2,700 calories per day for the four-week period during which anorexia was a problem. However, one should not completely ignore the possible effect of increased intake of food, which occurred at approximately the same time as protein was added to the diet of two of the subjects, and "pan" therapy to one.

*a. Physical performance.* The effects of the supplements described above, superimposed upon thiamine which was being added routinely, were not unequivocal. Subject E-3, to whom all supplements were given (plus new menus), exhibited an arrest of the progressive decrease in work output on the bicycle

ergometer, followed by a definite progressive improvement. Compared with the responses obtained in all other subjects, the improvement shown by this subject was relatively marked and rapid (fig. 1). In two subjects receiving protein plus thiamine and new menus, improvement in work output was not found, unless the absence of further decrease in work output was a sign of improvement. This arrest in the progressive drop in work output was not observed quite as quickly, however, in two subjects (E-6 and E-7) whose food intake increased because of the new menus, but who received no supplements other than thiamine (fig. 1). Post-exercise pulse rates in general diminished, although rather gradually.

There was no significant difference in the amount of improvement on the bicycle ergometer between those who did and those who did not receive protein, nor was there any in the post-exercise pulse rates following the step and treadmill tests, except in subject E-5. In the case of subjects E-6 and E-7 who received no further supplementation at this time, further deterioration occurred as evidenced by higher post-exercise pulse rates in subject E-6, and an inability to complete the treadmill test in subject E-7 (figs. 2 and 3).

*b. Psychomotor and psychologic response.* In general, there appeared to be some improvement in the psychomotor category during the supplementation program described for this period (figs. 5 and 6). It is again emphasized that caloric intake increased for all, and that the protein and/or crystalline vitamin supplements were superimposed on thiamine.

Ataxia was decreased in the subject receiving full supplementation, but this improvement was transient, subsequent scores again being high. A similarly transient improvement was noted in the subjects receiving protein. Subjects E-6 and E-7, whose overall deterioration in this test had been least, did not maintain the improvement previously noted with the addition of thiamine (fig. 4).

III. *Supplementation with Nicotinamide and/or Additional Foods, and Riboflavin.* At week 27, 300 mgm. of nicotinamide were given orally to all subjects, and then 150 mgm. orally for the next 6 days, followed by 12 mgm. per day thereafter. Subjects E-6 and E-7, who had received no crystalline supplementation except intravenous and daily thiamine beginning at week 19, now were given 40 grams animal protein per day. Subjects E-3 and E-4, who had shown what was considered to be the greatest decrement in physical performance, now received three additional foods daily—one egg at breakfast, 100 grams ground beef at lunch, and 225 grams fresh whole milk at dinner. At week 28, 15 mgm. riboflavin daily were given orally to all subjects for a period of seven days, and 1.5 mgm. per day thereafter. Subject E-4 was now also given daily the lesser-known B-complex vitamins listed in footnote 3, beginning at week 31. At this point, therefore, two of the experimental subjects were receiving complete crystalline supplementation (one of them had already been for some seven weeks) plus egg, meat and milk, while the remaining three subjects now lacked only the lesser-known B-complex supplements.

*a. Physical performance.* When this supplementation was superimposed on

that given previously, a definite overall improvement in physical performance occurred, as reflected by an increase in the duration of effort for each subject on the bicycle ergometer, and a decrease of resting and post-exercise pulse rate, in most instances, on the treadmill and step test (figs. 1, 2 and 3). The ability of subject E-7 to finish the first treadmill run reappeared. However, it was apparent that recovery was not altogether complete, since this subject was not yet able to finish the second run (fig. 3). In general, there was definite improvement in performance of all physical tests. Nevertheless, it is emphasized that the degree of recovery did not immediately reach levels of performance originally observed during the normal diet period.

b. *Psychomotor and psychologic response.* In general, the improvement observed as a result of previous supplementation was maintained, although there were occasional erratic performances by some subjects that were unexpected and are not easily explained. In most instances, scores as high as those found originally were made, although, as has been mentioned, the decrement observed in the psychomotor category had not been as great as in physical performance.

The increased degree of sway as measured by the ataxiometer presented an interesting discrepancy in that, while transient improvement had been noted with earlier supplementation, a rise in the amount of sway per unit of time was seen when the present supplements were superimposed (fig. 4). A similar rise was seen in the control subjects, and the significance of these findings is open to conjecture.

IV. *Luxurious Diet.* At week 36, a diet luxurious both in types of food (e.g.,—steak, frozen vegetables, pie à la mode, etc.) and in quantity (*ad lib.*) was fed to all subjects through week 39. There was, of course, a marked increase in the morale of the men. A striking further improvement in the duration of work performed on the ergometer was seen (fig. 1), and subject E-7 was now able to complete the second treadmill test for the first time in 13 weeks (fig. 3). However, no similar degree of improvement was found in resting or post-exercise pulse rates (figs. 2 and 3). Indeed, there were certain increases, exceeding any levels observed previously. A similar change was found in one of the controls, possibly implying that post-exercise pulse rate may not always be completely dependable as a measure of physical efficiency. In certain psychomotor tests, a decrement in performance was shown by some subjects (figs. 4 and 5) although, in general, the impression gained was that in this type of test the improvement noted heretofore was at least maintained. Several subjects, including one control, had unexpectedly high scores (i.e., increased sway) on the ataxiometer (fig. 4).

V. *Results other than Physical, Psychomotor or Psychologic Response.* 1. *Clinical symptoms and signs.* The buccal and tongue irritations and lesions occurring in the early stages had disappeared by now without treatment. The occasional appearance of a magenta tongue and lips in one subject during the supplementation period receded, but without reference to any specific therapy, as did the reports of heartburn and flatulence. The feeling of lassitude and languidness likewise disappeared, but rather gradually, so that it was difficult to ascribe this

change to the effect of any one nutrient. The scrotal lesion receded in those in whom it occurred, before the administration of large quantities of nicotinamide or protein. In retrospect, foot and leg pains were the most troublesome symptom, and appeared to become progressively worse in subjects E-3 and E-4, during the period of supplementation with the better known B-complex vitamins (i.e., thiamine, riboflavin and nicotinamide) and protein. Subject E-3 continued to report extremity pain and an inability to sleep soundly throughout the night even when supplementation with all the crystalline vitamins had been received for a considerable length of time<sup>10</sup>. When subject E-4 received the lesser-known B-complex vitamin supplements at week 31, the leg pain symptoms had not disappeared. Following the addition of this B-complex group, however, this symptom did gradually decrease in intensity and occurrence, and finally receded completely. Again, the individual variability was rather striking. The other three experimental subjects reported this symptom only infrequently, or after it had disappeared. We have therefore the situation of: (a) two of the subjects being affected by uncomfortable extremity pain which disappeared slowly upon liberal supplementation, and (b) the remaining three subjects finding this symptom to be a relatively minor difficulty even when supplementation was not complete.

Roentgenologic examination of the upper gastro-intestinal tract performed following complete supplementation revealed a tendency toward the return to normal motor function, although the experimental subjects still showed some of the findings which have been described as a "disturbed-motor function" associated with nutritional deficiency. Some of the subjects showed more improvement than others, and this improvement was observed both in gastric emptying time and in the decrease of segmentation of barium in the small bowel.

2. *Body weight.* Body weight decreased in the experimental subjects an average of 12 lbs. per man, including one subject who originally had been obese and whose decrease amounted to 17 lbs., and one other subject whose decrease was no greater than that of the controls. Each control subject lost approximately 4 lbs. After the new menus were added, the diet was again completely consumed and the body weight of each subject either rose or leveled off. The greatly increased caloric intake when the luxurious diet was given at week 36 resulted in a rapid increase in body weight in some subjects, including the two controls.

3. *Miscellaneous.* In several of the experimental subjects there appeared to be a slight decrease in the quantity of hemoglobin, the number of erythrocytes and the volume of packed red cells, all of which tended to increase in the later stages of full supplementation to the levels observed during the normal diet period. Thus, at certain points during the restricted intake, subjects E-4, E-5 and E-7 had levels as low as 12.5 grams of hemoglobin per 100 cc. of blood, four

<sup>10</sup> Because of the agreement between investigators and subjects that gross deficiency symptoms would not be permitted to develop, or if they did, that immediate efforts would be made to dispel them, no detailed attempt was made to determine which of the *individual* nutrients would mediate the disappearance of the leg and foot pain here described.

million erythrocytes per cubic millimeter, and a hematocrit volume of 32 cc. per 100 cc. All of these levels were lower than normal for young males, were lower than the individual normals originally determined, were lower than the lowest level found in the controls, and showed increases during supplementation period, so that in general, at the end of the experiment, the original levels had been regained.

Creatinine excretion dropped considerably at the beginning of the experimental diet period, and continued to decline more or less steadily except in the control subjects. A leveling off and a slight rise were found to occur whenever protein supplements were added, but no decisive rise towards the original individual normal occurred until the luxurious diet was fed. Detailed data are presented in a separate report (25).

Reference was made earlier to the decrease in plasma carotene that occurred in all 7 subjects when the experimental diet was fed. Vitamin A levels remained relatively constant, however. A rise occurred when the luxurious diet was fed, at which time the intake of preformed vitamin A was greater than in the experimental diet.

4. *Multiphasic personality tests.* Some of the most striking findings were obtained in the personality appraisal test which was given on five occasions as follows: (a) at the start of the experimental diet; (b) just prior to the beginning of supplementation; (c) when new menus were added, and subsequent to thiamine administration to all, protein to subjects E-3, E-4 and E-5, and nicotinamide and riboflavin to subject E-3; (d) subsequent to supplementation of all subjects with protein and the better-known B-vitamins, and subjects E-3 and E-4 with the lesser-known B-vitamins; and (e) during the third week on the luxurious diet. Figure 9 shows the results for one control subject and one experimental subject, the latter chosen as being representative for four out of the five.

At the outset, all seven subjects' personality inventory scores fell within the "normal" range. By the end of the 15th week, when the test was given the second time, the experimental subjects' scores had increased in the categories of hysteria, hypochondriasis and depression. No decisive changes were evident in the control subjects' scores. On the third test, it was found that the experimental subjects' scores then exceeded the upper limit of the normal range in the categories mentioned. Again, there was no marked change found in the control subjects. These findings are similar to those reported by Keys and co-workers (3). Return to normal limits occurred following supplementation, one of the interesting observations being that in three of the experimental subjects, the highest points were found at a period when thiamine had been administered for some length of time. As in the case of physical and psychomotor tests, improvement occurred rather slowly, but none the less definitely, when all supplements were provided. Return to normal scores was complete when the final test was given during the luxurious diet period (fig. 9).

5. *Sulfathalidine.* At week 33, one control subject (C-1) and one experimental subject (E-6) were given 4 grams of sulfathalidine daily. This was continued

for 17 days. Subjects E-5 and E-7, and subject E-3, received the same amounts for a shorter time, 10 and 8 days, respectively. Subjects C-2 and E-4 did not receive the drug. The purpose was to explore further the possibility that administration of a sulfonamide might provide some indication of the degree of dependence of the body upon vitamins produced in the intestine by biosyn-

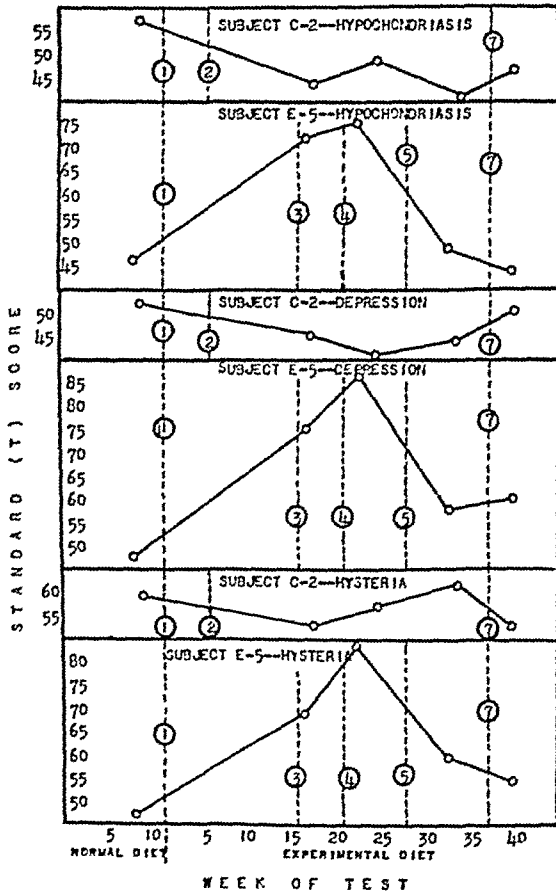


Fig. 9

Fig. 9. Personality inventory scores. The normal range of (T) scores is 30-70.

Fig. 10. Excretion of certain B-vitamins. Weeks during which sulfathalidine was administered are identified by filled circles.

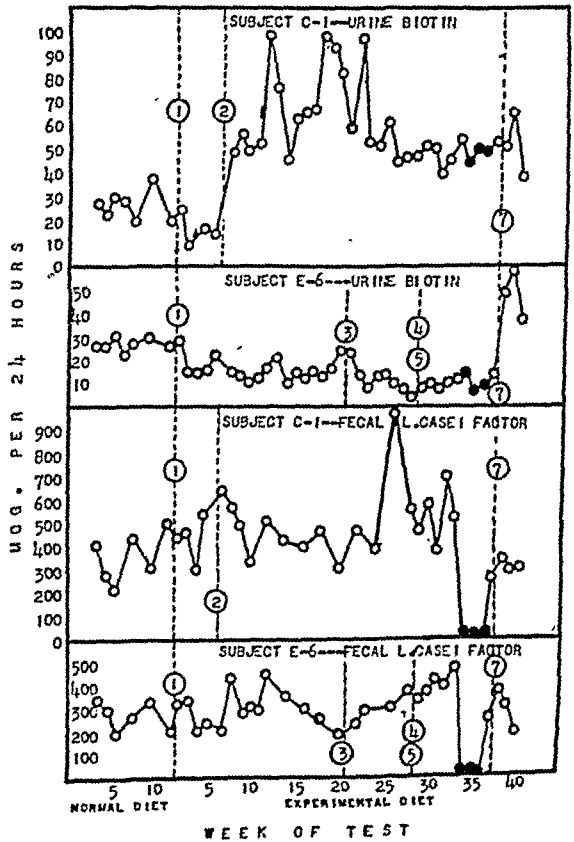


Fig. 10

Figs. 1-10 inclusive. Numbered vertical dotted lines designate the times at which dietary and supplementation changes were effected. These are described on pages 620 to 623 and were as follows: 1. Experimental diet started. 2. Full supplementation started. 3. Thiamine supplementation started. 4. Protein supplementation started. 5. Nicotinamide and riboflavin supplementation started. 6. Egg, meat and milk started. 7. Liberal diet and yeast started.

thesis. In addition we hoped to obtain data on the types of bacteria mainly responsible for production of different vitamins in the intestine. Evenson and co-workers have recently reported that the feeding of succinylsulfathiazole caused a decrease in the coliform and lacto-bacillus groups in feces of rats, and that the former tended to become re-established during continued administration of the drug (35).

We found that when sulfathalidine was administered, the fecal excretion of L.





would permit objective measurement of change. As time went on, we had occasion to consider the possibility of subjective influence upon even the most "objective" of the tests. Thus, resting pulse rates were found to be higher than normal. These may have been related primarily to a changed psychic state, such as hysteria, rather than to a decrease in physical efficiency or to a specific deficiency such as specific lack of thiamine. When tests of physical performance are used which depend more or less completely upon the personal desire of the subject to perform to his maximum capacity, then motivation becomes an important factor. Although it would appear likely that post-exercise pulse rate should indicate the physiologic extent of the effort, here again, the possibility of psychic influence enters. In this experiment it is not likely that these factors played a major rôle, for the subjects informed us at the end of the test that they always tried to beat their previous marks to the very best of their abilities. This statement itself indicates how important motivation might have been in subjects less desirous of co-operating and contributing to science. All of these possibilities influence the interpretation of results; however, because of the decrease in performance observed in *all* experimental subjects, the lack of similar findings in the controls, and the general although variable improvement noted with therapy, it is most unlikely that subjectiveness constitutes any major part of the explanation for the changes observed. On the other hand, the interesting possibility suggests itself that the very subjectiveness which workers in the field of human nutrition seek to exclude, may indeed be one of the important early changes resulting from nutritional inadequacy, with many of the changes eventually occurring in the physical and psychomotor aspects being mediated through a changed psychologic state.

An unequivocal answer as to which of the lesser-known B-complex factors are required in human nutrition is not obtainable from the data presented here. Certainly it appeared that improvement in performance on the bicycle ergometer was more rapid and permanent when the lesser-known factors were provided as a group together with thiamine, riboflavin, nicotinamide and protein. This difference was not found in the psychomotor category, however. It must be kept in mind that the addition of protein (or its component amino acids) may have introduced a "sparing" effect similar to that recently shown to occur with nicotinic acid (38, 39). In the clinical category, the refractory nature of extremity pains in two of the subjects is open to at least two alternative explanations: (a) these pains did not disappear until the lesser-known factors were added, or (b) the pains may have slowly disappeared had the only supplementation given been thiamine, protein, riboflavin and nicotinamide. We feel that the former is more likely to be correct in view of the opportunity for recovery given subject E-4, whose leg pains did not disappear after ample supplementation with the "better-known" factors. The fact that spontaneous remission of many of the clinical signs occurred does not necessarily dissociate those signs from B-complex deficiency as a cause-effect relationship. Such remissions have been noted frequently in clinical cases of B-complex deficiency disease.

Certain of the biochemical data pose new questions and point the way for further investigation. One of the most striking findings was that of a relatively high level of excretion of vitamins in the feces throughout the period of restricted intake. It seems rather remarkable that the body should show signs of deficiency while large amounts of the nutrients which it lacks are contained in the feces. However the vitamin levels of the feces undoubtedly assayed high because of the vitamin content of living and dead bacterial cells. Such vitamins would not be available, particularly if they were in the form of a complex protein normally requiring hydrolysis. It is not likely that this chemical process can or does occur in the lower level of the intestine where the greater portion of synthesis is believed to take place. On the other hand, there is no absolute evidence from our work that some absorption did not actually occur, but this seems questionable, particularly in those instances where urinary excretion levels were zero, or nearly so, during the course of the experiment.

Reference was made earlier to the unusually low values found for beta-carotene, in spite of the increased intake of that substance during the experimental diet period. At least two explanations of this finding are possible: (a) a decreased absorption of beta-carotene—although this would seem to be unlikely since the plasma vitamin A levels decreased only slightly, or (b) because of the moderate decrease in preformed vitamin A in the experimental diet, a greater per cent of the beta-carotene was converted in order to maintain the status of the former, thus causing lower plasma levels of beta-carotene. At best, however, the finding merely suggests the need for further investigation.

One question arose at the outset concerning the "completeness" of crystalline supplements for the controls. It was recognized that as-yet-unknown nutrients probably contained in liver or yeast extract were not provided in the crystalline supplements. However, in view of the overall maintenance of physical and psychomotor performance (or improvement in certain instances) by the control subjects, the relatively slight drop in body weight, and the high morale and subjective feeling of well-being which the controls exhibited throughout, it is believed to be unlikely that as-yet-unknown nutrients in amounts greater than provided by the experimental diet play an important rôle in the nutritional status of young men for the length of time reported upon here. Nevertheless, there is still the possibility that such nutrients could be provided through bacterial synthesis in the intestine, and subsequent absorption.

Last but far from least in practical importance was the marked slowness in recovery of ability to perform the amount of work within the original capability of the individual, even when complete supplementation was given. No portion of the recovery curve was steep, until the luxurious diet was fed. There was no "magical" effect of any vitamin, nor of protein in the form of calcium caseinate. Recovery following supplementation with vitamins and protein was not immediate, and restoration of efficiency in the depleted individual was not complete until rehabilitation in the physiologic sense had proceeded for some time. The striking response in work performance elicited at the time of feeding the

luxurious diet serves to emphasize the importance of feeling well fed, as well as being physiologically well fed, if optimal rapid response is to be obtained.

#### SUMMARY AND CONCLUSIONS

Following an initial control period of 12 weeks on a normal diet, seven normal males consumed daily for 35 weeks a diet containing approximately 3000 calories and restricted levels of B-complex vitamins and protein averaging as follows: thiamine 0.50 mgm., riboflavin 0.30 mgm., nicotinic acid 5.8 mgm., L. casei factor 23 mcg., pyridoxine 1.1 mgm., pantothenic acid 1.1 mgm., biotin 19 mcg., and protein 45 grams (94 per cent non-animal in origin). Corn comprised 27 per cent of the total caloric intake. After five weeks of the restricted intake, two of the subjects were chosen as *controls*, and thereafter received supplements of nutrients in amounts which made their total intakes equal to or slightly higher than the levels during the control period. Placebos that were identical in appearance were received by the remaining five subjects who are referred to as *experimental* subjects. At the end of 15 weeks of the experimental diet, three of the five experimental subjects were supplemented with thiamine. The remaining two experimental subjects were similarly supplemented three weeks later. Thereafter, additive supplementation with protein, riboflavin, nicotinic acid and lesser-known B-complex factors (folic acid, pyridoxine, pantothenic acid, and biotin) was accomplished at the end of varying periods of time. One subject received the added nutrients as a complete group, another subject received them in two steps, and the remaining three experimental subjects in a series of step-wise additions. At the end of 20 weeks of this supplementation schedule, the experiment ended with a three-week period of a luxurious diet. The total elapsed time, including the control period, was 51 weeks.

At the end of 15 weeks on the experimental regime, definite decrease in the amount of work performed on a bicycle ergometer and elevation of resting and post-exercise pulse rates had occurred in all experimental subjects, and some were unable to complete step and treadmill tests which they had been able to perform with relative ease during the control period. Performance on these tests by the control subjects was, on the contrary, either moderately improved, or unchanged from that observed during the normal diet period. Changes in the psychomotor category were less clear-cut, although they were judged to have occurred in view of the moderate improvement that appeared subsequently when the experimental subjects received supplementation.

Incontrovertible stigmata of specific deficiencies were not observed in the experimental subjects although a number of suggestive signs were present in varying degrees. These included superficial mouth ulcers, magenta tongue, angular stomatitis and cheilosis, mild erythematous glossitis, scrotal dermatitis, and a tendency towards disturbed motor function of the small bowel. Most of the signs were mild, some of them regressed apparently spontaneously, and not all of them occurred in all experimental subjects. Body weight declined only moderately. The subjects reported the following symptoms: lassitude, in-

somnia, sore mouth, pyrosis, anorexia, nausea, vague paresthesias, and severe cramping pains of the extremities. Of these, the extremity pains were persistent in two subjects, even after supplementation had been received for some time. Personality appraisal scores (Minnesota Multiphasic Personality Inventory) indicated a change toward hysteria, depression and hypochondriasis. With the exception of superficial mouth ulcers, and angular stomatitis (following dental treatment) all of the foregoing signs and symptoms were absent in the control subjects.

In the case of the experimental subjects, urinary excretion and load test levels of the B-complex vitamins were low, but the levels of vitamins in the feces were relatively unchanged from those found in the normal diet period. Positive nitrogen balance was maintained throughout. The excretion of free l-tryptophane did not decrease from that found in the normal diet period.

When the experimental subjects received supplementation with crystalline nutrients, their urinary vitamin excretion levels soon returned to, or exceeded, normal. Improvement in physical performance tests occurred gradually, rather than immediately. No evidence was obtained pointing to a "magical" effect of any one nutrient, and improvement in physical performance was more rapid in subjects receiving the complete group supplementation than in those receiving stepwise additions of nutrients. Improvement also occurred in certain psychomotor tests, although fluctuations tended to make decisive interpretation difficult. Re-appraisal of personality following supplementation indicated gradual return to normal scores. Improved motor function of the small bowel was also found. The lower extremity pains, which persisted in two subjects, disappeared slowly at first as B-complex supplementation was received, but by the end of 20 weeks of supplementation these symptoms had subsided almost completely. All other clinical signs and symptoms which had not regressed earlier now disappeared, but it was difficult to associate their disappearance with the addition of any one nutrient. Thiamine in particular was not effective in dispelling the symptoms or improving the physical and psychomotor scores. Striking improvement in the duration of work on the bicycle ergometer was obtained during the last three weeks of the experiment when a luxurious diet was fed.

Administration of four grams per day of sulfathalidine from experimental weeks 33 to 35 resulted in a decrease in the number of coliform organisms in the feces. This was associated with a coincident decrease in *L. casei* factor and biotin content of the feces. Fecal content of thiamine was decreased slightly in three subjects, and two of these three subjects also excreted less riboflavin and pantothenic acid than they had immediately preceding the administration of sulfathalidine. No decisive reduction in *urinary* levels was found, however, in either the control or experimental subjects receiving the sulfathalidine. No change in physical or psychomotor performance was found during the period of sulfathalidine administration.

Throughout the 35 experimental weeks, the two control subjects showed no

decrease in work performance, physical and psychomotor efficiency, or personality appraisal while consuming the experimental diet plus supplements. No signs or symptoms suggestive of nutritional lack were observed. Since definite changes in all these categories were observed in the five experimental subjects, and these changes were reversed upon adequate supplementation, it is concluded that the dietary regime was not adequate for young men 23-28 years of age, at a moderate caloric expenditure level. The salient points of this average daily dietary regime were: (a) caloric intake of approximately 3000 calories, and (b) a combination of the following: 0.50 mgm. thiamine, 0.30 mgm. riboflavin, 5.8 mgm., nicotinic acid, 45 grams protein (mostly non-animal in origin), and lower than normally encountered levels of the following lesser-known B-complex vitamins: folic acid, pyridoxine, pantothenic acid and biotin.

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## BLOOD FLOW IN THE BRONCHIAL ARTERY OF THE ANESTHETIZED DOG<sup>1</sup>

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The hemodynamics of the bronchial circulation are of unusual interest for several reasons. First, the bronchial artery is the nutrient supply to all the intrapulmonary tissues, including blood vessels and nerves, and as pointed out by Daly (1, 2) a suboptimal flow could produce far-reaching effects on the function of the lung. Secondly, the bronchial flow is capable of invading the pulmonary circulation directly via anastomotic capillaries on the respiratory bronchioles and indirectly through the pulmonary veins into which drains much of the bronchial flow (see fig. 2 below). Consequently, the aerated pulmonary vein blood is continuously contaminated with bronchial venous blood and the pressures in the pulmonary circulation can be modified by changes of flow and pressure in this aortic branch. By either of these mechanisms abnormal flow resistance in the bronchial artery might upset the balance between hydrostatic and osmotic forces in the pulmonary capillaries and promote pulmonary edema, particularly the paroxysmal type which develops suddenly without obvious abnormality in the cardiovascular system, lung membranes or blood constituents. In the literature, as reviewed by Luisada (3) and Schlesinger (4), a neurogenic cause of paroxysmal pulmonary edema has been emphasized repeatedly, but there has been no factual explanation of the mechanisms whereby nerve impulses or their absence are translated in edema. Changes in vasomotor activity of the pulmonary vessels are an unlikely cause since these fibers have been studied extensively without mention of edema (5), although the edema following bilateral vagotomy is a possible exception to this generalization (6). The edema in heart-lung and isolated lung preparations is ascribed to toxic factors in the perfusate (7, 8, 9). Existing evidence indicates that the vasomotor control of the pulmonary system is relatively undeveloped (5). This leaves the vasomotor innervation of the bronchial circulation as the most obvious possibility among neurogenic influences which could cause paroxysmal pulmonary edema; a further argument is the fact that the intrapulmonary bronchial artery is adequately supplied with nerve fibers (10, 11).

In order to decide whether the bronchial flow could overtax the drainage capacity of the pulmonary veins and raise pulmonary capillary pressure to the point of causing edema, two lines of evidence are required: One, the maximal flow and pressure gradient of the bronchial artery blood which drains into the pulmonary veins and the other, the nature and degree of vasomotor control of the bronchial vessels.

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The nearest approach to the required data was found in the studies by Daly and associates (12, 13, 14) who perfused the pulmonary and bronchial vessels of dogs with defibrinated blood by separate pumps. They observed that *a*, stimulation of any of the nerves to the lung resulted in vasoconstriction in the bronchial artery, if there was any response; *b*, an increase in flow and pressure in the bronchial artery system usually was associated with a rise in pressure in the pulmonary artery, but sometimes there was no change or a decrease; *c*, variations in pulmonary artery pressure and flow influenced the fraction of bronchial artery blood draining into extrapulmonary veins; *d*, the bronchial vessels were constricted by adrenalin and dilated by histamine. Miyaki (15) reported that spirals of bronchial arteries *in vitro* behaved like systemic arteries in response to several drugs. However, the significance of all these findings in relation to hemodynamic problems *in vivo* is open to question and no data were discovered on the peak quantities of blood carried by the bronchial artery.

The practical aspects of this general problem received added emphasis during the war years. The high incidence of pulmonary edema in cases of head injury (4) was of immediate concern to the medical services as a whole. In aviation medicine the admixture of venous blood with the arterialized pulmonary vein blood was important in certain theoretical considerations and the threat of chemical warfare gave impetus to better understanding of all causes of pulmonary edema. The data below were secured in an effort to assess the flow capacity and vasomotor activity of the bronchial artery in the etiology of pulmonary edema.

**METHODS.** The experiments were carried out on dogs under conditions as close to normal as possible, but the anatomic relations of the vessel necessitated extensive dissection and complete anesthesia. By preliminary dissection and injections of more than 75 dogs, the right posterior bronchial artery, a branch of the fifth or sixth right intercostal artery, was found to be the chief right bronchial artery in about 60 per cent of instances; in the remainder, the posterior bronchial artery was absent or minute and the bronchial blood supply came via branches from the internal mammary and other arteries. In these experiments dogs weighing from 10.6 to 19.0 kgm. were anesthetized by pentobarbital (32.5 mgm. per kilo intravenously) or chloralose (50 mgm. per kilo intravenously following morphine 5 mgm. per kilo subcutaneously) with small supplements as required to maintain a light surgical level. Strict hemostasis by ligation, cautery and thrombin were necessary because subsequent heparinization was required for the flow measurements. Artificial respiration from a Starling pump via a tracheal cannula was employed and a stream of moist oxygen was also passed through a tracheal catheter at the rate of 2 to 3 liters per minute. The right posterior bronchial artery was approached by resecting the posterior portion of the sixth right rib using the posterior perforating branch of the sixth intercostal as the landmark. The fifth intercostal vein, and sometimes the sixth also, was severed and the azygos vein retracted anteriorly to expose the bronchial artery. The parent intercostal was then dissected free as far as the sympathetic chain and ligated; the only remaining exit for blood from it was the bronchial artery (fig. 1).



Blood flow through the vessel was measured by means of a small (1.9 cc.) bubble flowmeter (16) encased in a jacket through which water at 38°C. continuously circulated. Heparin (Abbott) was injected intravenously in dosage of 366 Toronto units per kilo, repeated every hour; with this excessive dose no signs of fibrin or platelet deposition were seen (17, 18). Cannulae then were inserted into the intercostal and femoral arteries and after the system was filled with fresh blood the intercostal was tied off centrally. The supply of the bronchial artery thus was thrown onto the flowmeter without interrupting flow into the artery. The blood pressure in the femoral artery was found to equal that in the intercostal.

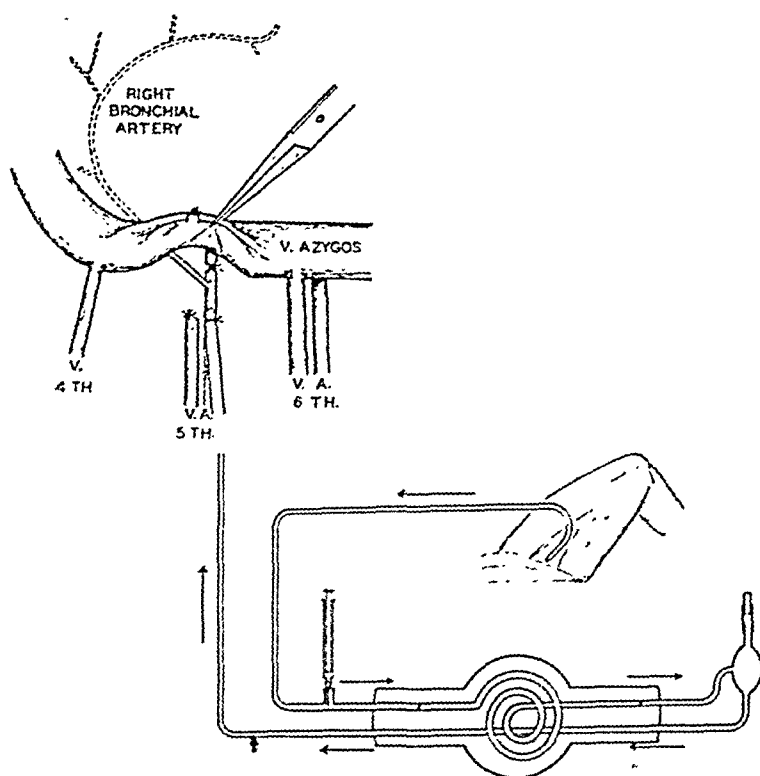


Fig. 1. Diagram showing the usual origin of the right posterior bronchial artery of the dog, and the circuit with meter by which flow was measured.

In about two-thirds of the experiments the dogs were maintained throughout on artificial ventilation supplemented with oxygen. The pump was adjusted at a rate of 20 per minute so that the lungs made full contact with the chest wall and moved the diaphragm slightly on inflation, and retracted from the chest wall on deflation only slightly. In the remaining experiments, the chest was subsequently closed over a metal plate provided with an air-tight slot for the tube connecting the meter to the intercostal cannula; spontaneous breathing was present during the flow measurements thereafter.

For nerve stimulation, the vagi were exposed in the neck and the sympathetic fibers by resection of the second right rib posteriorly. Only the strands arising from the mid-portion of the mesial margin of the right stellate ganglion were

isolated and used; it is very doubtful that all the sympathetic fibers to the bronchial artery were contained in these strands (19). The stimulating currents from a Harvard inductorium or thyatron stimulator were weak or, in a few instances, moderate. No signs of current escape were noted; shielded electrodes were used when the nerve could not be lifted free.

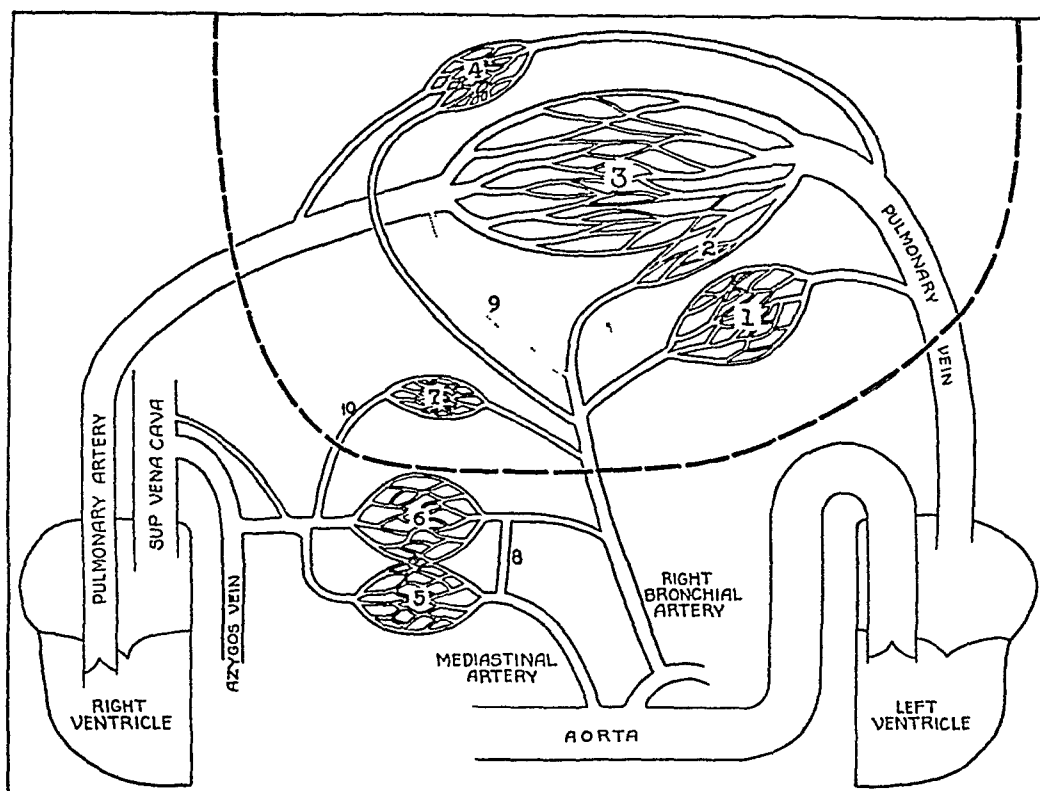


Fig. 2. Schematic diagram of the vascular connections of the right posterior bronchia artery in the dog. The heavy dashed line separates intra- and extrapulmonic portions. 1: Capillary bed of the general intrapulmonic flow. 2: Capillary bed on respiratory bronchioles showing fusion with 3: Alveolar capillary bed supplied by the pulmonary artery. 4: Visceral pleural capillaries supplied by either or both systems; drainage in either case as shown. 5: Capillary bed of a mediastinal artery fusing with 6: Capillary bed of extrapulmonic branches of the bronchial artery. 7: Capillary bed of intrapulmonic bronchial artery branches proximal to first 2 or 3 dichotomies and in perihilar pleura. 8: Collateral arterial channels. 9: Once claimed collateral connection now recognized as *vasae vasorum* on pulmonary arteries from bronchial. 10: True bronchial veins.

Arterial blood pressure was recorded by a mercury manometer from the left carotid artery. Drugs were injected both intravenously (femoral) and intra-arterially through a valve on the distal end of the flowmeter. All solutions were prepared in Ringer's fluid and injected at body temperature.

The flow measured in the posterior bronchial artery did not give direct values for intrapulmonary bronchial flow since *a*, this artery is not always the sole source of blood to the bronchial circulation, and *b*, some of the measured flow supplies extrapulmonary tissue. Figure 2 which is based on the data of these experiments and of other investigators (20, 21, 22, 23, 24) schematizes the ana-

tomic background. The first difficulty was obviated by adding a solution of T-1824 dye to the inflowing blood just before killing the animal and considering only those experiments in which all the bronchial mucosa, hilar area, and adjacent zone of mediastinal, tracheal and esophageal tissue were stained; such staining indicated minimal or no admixture of extraneous blood with the metered flow. The other difficulty necessitated an estimation of the distribution of measured flow between the intra- and extrapulmonary beds; these data are given below.

RESULTS. 1. *Fractionation of right bronchial artery flow between intra- and extrapulmonary tissue.* This was accomplished by an adaptation of the  $P^{32}$  technique devised by Hevesy *et al.* to measure red cell volume (25). The cells in a 5 cc. sample of the dog's own blood were labeled with  $P^{32}$ , mixed with a small amount of solid T-1824, and added to the blood flowing into the bronchial artery. When the mixture was judged to have filled the capillaries, the animal was killed abruptly by intravenous injection of saturated  $MgSO_4$  solution. The origin of the bronchial artery and the bronchi were ligated, and a measured volume of left atrial blood set aside for analysis. The left atrium was opened and the pulmonary bed was perfused gently with warm saline via the pulmonary artery until the effluent was moderately clear. The lungs then were cut free flush with their hilar faces, weighed and placed in separate Kjehldahl flasks. All blue-stained mediastinal tissue except the bronchial artery to its first main branch and its accompanying vein were excised for analysis. The tissues and blood were wet ashed, diluted to constant volume, and their radioactivities determined in a constant geometry  $\beta$ -ray counter.

Since the radioactive cells were added while flow and pressure in the bronchial and pulmonary circulations were at normal levels, the distribution of radioactivities is assumed to be that of blood flowing into these areas in the intact animal. The procedure is intended to determine the relative distribution of the metered flow, not absolute bed area. No claim is made for high accuracy, but the constancy of the data in table 1 indicates that reliance may be placed in them. At least this seems the most physiological method yet employed for obtaining this type of information.

Six experiments were performed; three animals were ventilated artificially and three breathed spontaneously. According to the results shown in table 1, an average of 69 per cent of the blood flowing through the right bronchial artery went to the right lung, and 31 per cent to the mediastinal structures. Recirculation of radioactive cells from both right and left ventricles (see fig. 2 and last two columns of table 1) was consistent with the overall accuracy of the method. This fraction agrees with the finding of Berry and Daly (12) that at least two-thirds of the blood perfused via the bronchial circulation passed to the pulmonary veins when both bronchial and pulmonary arteries were perfused at normal pressures.

This partition factor, applied to the flow values of the right bronchial artery, permits calculation of the total amount of blood discharged from the bronchial arteries into the pulmonary veins in animals whose intrapulmonary flow from vessels other than the right posterior bronchial artery is negligible, assuming

that the bronchial flow into the left lung is proportional to that into the right in terms of relative lung weights. In 30 normal animals the weight of the right lung was found to average 1.28 times the left. Thus, the observed right bronchial flow times  $[0.7 + 0.7 (0.8)]$ , or 1.26, would approximate the total flow discharged into the pulmonary veins in a given dog per minute.

2. *The "normal" flow.* The following data are presented as the closest approximation now possible to bronchial blood flow in lightly anesthetized, heparinized dogs under the stipulated experimental conditions.

TABLE 1

*Distribution of radioactivity following injection into the right bronchial artery of red cells labelled with  $P^{32}$ . Ratios computed from recorded counts per minute*

DOG	VENTILA- TION	RT. LUNG	MEDIASTINUM	RT. LUNG	MEDIASTINUM
		RT. LUNG + MEDIASTINUM	RT. LUNG + MEDIASTINUM	LT. LUNG	LT. ATRIAL BLOOD/CC.
A	Spont.	0.804	0.196	14.6	61.1
B	Spont.	0.665	0.335	16.4	158.0
C	Spont.	0.783	0.217	13.4	70.4
D	Pump	0.635	0.365		
E	Pump	0.730	0.270	59.7	
F	Pump	0.535	0.465	21.8	653.0
Average.....		0.692	0.308		

TABLE 2

*Blood flow through the right posterior bronchial artery of 50 dogs ventilated by pump*

	MEAN FLOW $\pm$ STANDARD DEVIATION	COEFFICIENT OF VARIATION	OBSERVED RANGE
	cc./min.	per cent	cc./min.
Average normal flow.....	$4.8 \pm 2.54$	53	1.0-12.0
Average normal flow per 100 mm. Hg blood pressure.....	$3.9 \pm 1.98$	51	0.9- 9.3
Maximal normal flow.....	$6.6 \pm 4.19$	63	1.5-18.5
Minimal normal flow.....	$3.1 \pm 1.91$	62	0.5- 8.2

The findings in 50 dogs under standard artificial ventilation are summarized in table 2. The average normal flow was estimated from the graphed or tabulated data since the flow frequently was labile (see section 5 below). The maximal normal flow was the highest spontaneous flow observed and gives an indication of the average lability in the direction of increased flow. The minimal normal flow, although it depends primarily on an arbitrarily defined lower limit of normal, lends confidence to the average normal value. These data were taken from the beginning phases of observation before anything was done to alter flow and pressure.

In general, flow was highest at the beginning of observation; subsequently it declined with time even if no experimental procedures were carried out. This

was shown in 12 control experiments of  $1\frac{1}{2}$  to 8 hours' duration (5 under pentobarbital and 7 under chloralose-morphine anesthesia) in which the animals were undisturbed except for the hourly dose of heparin and supplements of anesthetic. In all experiments blood pressure also decreased; at least a partial cause was slow loss of blood, as 100 to 400 cc. of blood invariably were found in the thoracic cavity at autopsy. In 7 of the 12 control experiments flow definitely decreased some 30 to 60 minutes before pressure began to fall. In the terminal phase the flow held to a rather constant low value regardless of further changes of pressure. Since the decrease of flow was asynchronous and out of proportion, quantitatively, with fall in pressure, the peripheral resistance must have increased. A pre-mortem dye injection and careful autopsy discredited embolism, edema,

TABLE 3

*Comparison of bronchial artery flows in 16 dogs when ventilated by pump and when breathing spontaneously*

	MEAN FLOW ± STANDARD DEVIATION	COEFFICIENT OF VARIATION	OBSERVED RANGE
	cc./min.	per cent	cc./min.
Average normal			
Pump.....	6.4 ±2.98	47	2.0-12.0
Spont.....	5.6 ±4.32	77	0.9-19.0
Average normal per 100 mm. Hg B.P.....			
Pump.....	5.3 ±2.03	38	1.9- 9.3
Spont.....	4.6 ±3.23	70	0.9-14.1
Maximal normal			
Pump.....	8.6 ±5.06	59	1.5-18.5
Spont.....	7.9 ±6.04	76	0.9-26.7

aberrant vessels, or mechanical influences as causes of diminished flow, and therefore vasomotor influences are the remaining possibility.

The consistency of the data of table 2 was not improved by expressing the individual flows in terms of existing blood pressure, surface area, or body-, lung-, or heart weights of the respective animals. In no instance were the distributions normal and there was little central tendency except with excessively large groupings. No lines of regression were detectable in scatter graphs except that for blood pressure versus estimated normal flow and this was about 8 cc. wide at normal pressure levels. Statistical treatment of the data therefore was not warranted. Since the first half of the experiments gave essentially the same averages as the second half, there was no proficiency bias. Vasomotor influences superimposed on anatomic variations of vessel capacity seem the most likely cause of the excessive dispersion.

As a closer approach to normal conditions, sixteen dogs were transferred from pump ventilation to spontaneous breathing, with the effects on flow as summarized in table 3. Since an interval of 40 to 100 minutes separated the two

sets of measurements, the spontaneous decrease of flow with time seen in the control experiments probably accounts for much, if not all, of the observed differences. Actually the flow increased in 4 of the 16 dogs, and in 2 the increases were large. The flow of 26.7 cc./min., the peak of a spontaneous variation, was the highest encountered under any circumstances. The coefficients of variation in table 3 and the scatter graphs indicate that the dispersion of flow was not reduced under spontaneous breathing. No systematic or significant change of flow seems to have resulted from this closer approximation to the normal state.

3. *The maximal flow.* In order to estimate the ultimate flow capacity of the bronchial artery, a major purpose of these experiments, a maximally effective dose of a vasodilator drug, theophylline ethylenediamine or mecholyl, was added to the blood entering the artery. In 27 experiments, table 4, the average of this flow was 2.0 to 2.5 times the average normal flow. The largest multiple was

TABLE 4

*The maximal induced flow through the right bronchial artery of 27 dogs ventilated by pump compared with their maximal normal and average normal flows*

	MEAN FLOW ± STANDARD DEVIATION	COEFFICIENT OF VARIATION	OBSERVED RANGE
	cc./min.	per cent	cc./min.
Maximal induced flow.....	10.6 ±5.4	50	2.9-26.2
Same per 100 mm. Hg B.P.....	8.4 ±3.2	38	3.8-18.3
Maximal normal flow.....	5.3 ±4.0	75	0.9-18.1
Same per 100 mm. Hg B.P.....	4.4 ±2.6	58	1.1-10.4
Estimated normal flow.....	4.3 ±2.5	57	1.0-12.0
Same per 100 mm. Hg B.P.....	3.5 ±1.8	52	0.8- 7.2

7.2, an increase from 0.9 to 6.45 cc./min.; in general, the higher the existing flow the smaller was the multiple of increase. A fraction, of the order of 20 per cent, of the increase is attributable to the pseudo-dilator effects of the saline diluent (section 7 below). The largest induced flow, 26.2 cc./min., was close to the highest recorded flow, 26.7 cc./min. Thus a reasonable estimate of the maximal flow capacity of the right bronchial artery is 25 to 30 cc./min., some 5 to 6 times the average normal flow. This estimate multiplied by the correction factor, 1.26 (section 1), gives 30 to 40 cc./min. as the maximum total drainage into the pulmonary veins from both right and left bronchial arteries in dogs of this size.

4. *Relation of bronchial blood flow to cardiac output.* In order to obtain the desired information on the relative amounts of blood carried by the pulmonary and bronchial systems, cardiac output was measured by the direct Fick method in 6 dogs breathing oxygen spontaneously. Mixed venous blood was drawn through a fine rubber catheter introduced into the right ventricle; its position was checked at autopsy. Samples of arterial and mixed venous blood, collected simultaneously, were analyzed in duplicate by the manometric Van Slyke tech-

nique. Oxygen consumption was measured for a period of 8 minutes or more by a Jackson spirometer calibrated *in situ* in each experiment. The normal output for these dogs averaged 1680 cc./min. (range 730 to 3200); this figure is within the ranges reported by others for dogs of this size (26, 27, 28, 29).

The first line of table 5 shows the average flows during 20 output determinations made at all stages of the experiments and includes the effects of hemorrhage, ephedrine intravenously, and gelatin infusion. The wide dispersion of the data is due in part to this inclusiveness. Both the highest and lowest spontaneous flows ever observed occurred in this group of dogs. Calculation of the flows in terms of blood pressure did not reduce the dispersion, but when the

TABLE 5

*Relation of right bronchial artery blood flow to simultaneous cardiac output in 6 dogs breathing spontaneously*

	MEAN FLOW ± STANDARD DEVIATION	COEFFICIENT OF VARIATION	OBSERVED RANGE
	cc./min.	per cent	cc./min.
1. Average high-low flow at time of C.O. determination (20 samples at all phases).....	4.2 ±4.8	116	0.6 -26.7
2. Same per 100 mm. Hg B.P.....	3.2 ±3.6	112	0.8 -15.6
3. Same in per cent of cardiac output....	0.23 ±0.16	70	0.03- 0.83
4. Same per 100 mm. Hg B.P. in per cent of cardiac output.....	0.21 ±0.12	57	0.04- 0.49
5. Averaged high-low flow at time of C.O. determination (11 samples during initial normal phase).....	6.4 ±5.9	92	1.2 -26.7
6. Same per 100 mm. Hg B.P.....	5.3 ±4.2	79	1.7 -15.6
7. Same in per cent of cardiac output....	0.34 ±0.13	38	0.11- 0.83
8. Same per 100 mm. Hg B.P. in per cent of cardiac output.....	0.30 ±0.11	38	0.15- 0.49

flows, absolute or per unit pressure, were expressed as per cent cardiac output, the consistency of the data improved considerably. Inspection of the plots of output against flow showed that the 11 measurements during the initial "normal" phase displayed a linearity: the flow decreased with decrease of cardiac output. These selected flow data are shown in the lower half of table 5. The dispersion of these flows was greatly reduced by relating them to their simultaneous cardiac outputs. This is regarded as significant because the Y intercept is neglected in such a procedure and it probably is not zero. The last line of table 5 indicates that the blood pressure was an important factor in these flows only insofar as it was a function of cardiac output. The remaining flows showed no relation to cardiac output. The ratio of flow to cardiac output consistently decreased with time, but irregularly after the initial phase; the cardiac output decreased relatively less than the flow. Special interest attaches to the

maximal fraction of cardiac output attributable to bronchial artery drainage into the pulmonary veins. This was observed to be 0.58 per cent of the cardiac output for the right bronchial flow alone, or 1.02 per cent for total bronchial flow. The flow used for this computation, 26.7 cc./min., appears a truly maximal value (c.f. section 3). Lines 3 and 7 of table 5 show that ordinarily the bronchial fraction of cardiac output is much smaller, 0.45 per cent or less, but even the 1 per cent figure appears insufficient to tax the capacity of the pulmonary circuit.

5. *Spontaneous variability of bronchial blood flow.* Only 8 of the 35 dogs, in which observations were sufficiently protracted, showed essentially constant bronchial flows. In 15 the variations were sporadic and/or relatively small, 0.5 to 2.0 cc./min. In the remaining 12 the variations were larger and more frequent; 8 of the 12 showed at some time a cyclic variation in flow like that illustrated in figure 3. These always broke up after a time into sporadic variations.

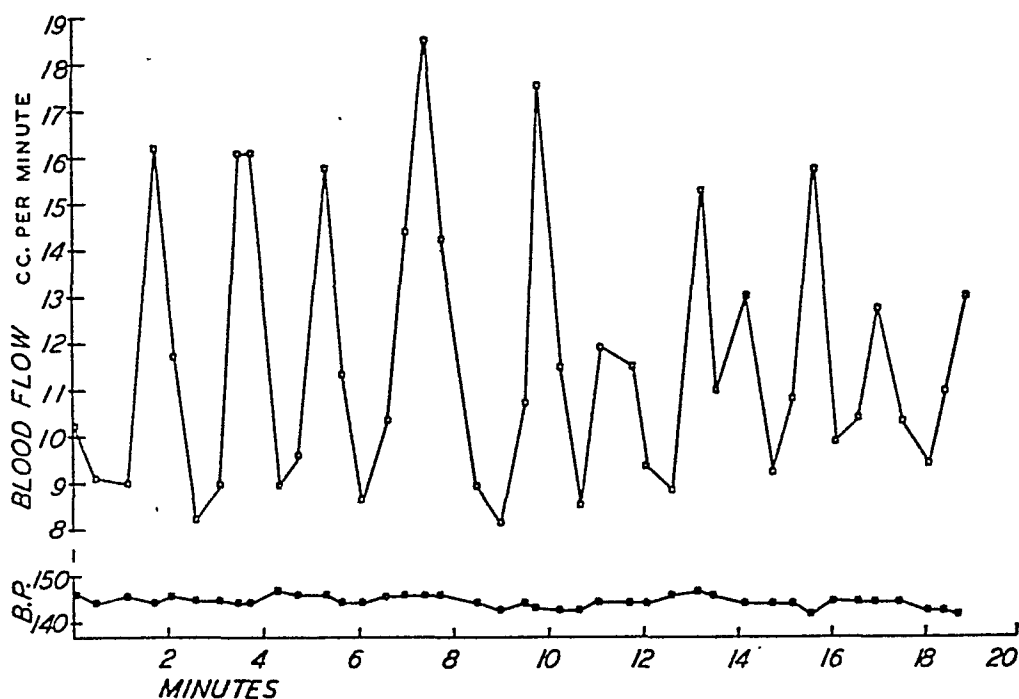


Fig. 3. Example of spontaneous variations of bronchial artery blood flow in a dog ventilated by pump; at the right is beginning break-up into sporadic variations.

The variability usually was most pronounced in the early part of the experiment and became less frequent and smaller as the flow decreased. The sporadic variations generally began and ended abruptly, but sometimes they took the form of a surge. The vast majority were in the direction of increased flow but instances of temporarily decreased flow were encountered. At some point in 5 experiments the flow was observed to stop and start up again alternately, and then return to its previous level.

The cause of the spontaneous variations was not ascertained: They had no



consistent relation to blood pressure; pressure changes, if present, were as often opposed to as consensual with the flow change and in any case were small. They occurred under spontaneous and pump ventilation. Bilateral vagotomy or section of the fibers from the stellate ganglion did not abolish or prevent them. They continued after increasing the depth of anesthesia; an acute 200 cc. hemorrhage or discontinuing the extra oxygen supply did not cause them to cease. Drug actions superseded them but only temporarily. They were not associated with any visible movements of the larynx, esophagus or other parts of the body.

The most probable remaining causes appear to be *a*, bronchoconstriction or bronchodilatation, and *b*, vasomotor activity in either the bronchial artery radicles or in the vessels with which it has visibly rich collateral communications. There was no evidence of changed bronchial diameter in the graphic record of breathing (from a side tube on the tracheal cannula), but because the tambour recorder would have registered only intense changes, the possibility of local areas of bronchodilatation getting into phase is not ruled out. Bronchoconstriction, however, is an unlikely cause since it should have reduced the bed area to give only dips below the sustained level; the same applies to esophageal spasm.

The flow capacity of the bronchial collaterals was estimated by applying the principles outlined by Green *et al.* (30) for such a system. The femoral supply to the meter was clamped off (fig. 1) and the meter opened so that the injected bubble flowed backward; this flow was against an unavoidable hydrostatic head of 12.5 mm. Hg which nevertheless is smaller than capillary resistance (31). Under these circumstances, the collateral flow (22 trials in 12 dogs) averaged 75 per cent of the existing direct flow; in only 4 instances was the collateral greater than the direct flow, and the largest was less than 25 per cent greater. The collateral flow decreased more or less in parallel with the decrease in direct flow as the experiment continued. Since the collateral flow under these favorable conditions was not greater than the direct flow, it is unlikely that changing collateral flow was responsible for a significant fraction of the observed spontaneous variations.

These findings indicate that the source of the variations is to be referred to the bronchial arteries themselves. They also demonstrate the error inherent in conclusions based on experimental ligation of the bronchial arteries near their origin (32).

6. *The effects of nerve stimulation and section.* These were studied in 25 animals using the cervical vagus (vagosympathetic trunk) or the accelerator strands from the stellate ganglion. The findings are grouped in table 6 where the effects are listed as vasoconstriction or vasodilatation. The former represents a decrease of flow while blood pressure remained constant or rose, and the latter an increase of flow while blood pressure remained constant or fell. It is evident that the vagus carries predominantly dilator impulses, whereas the sympathetic strands convey constrictor impulses. Examples of each type of response are shown in figure 4. Both responses showed latency and after-effects of variable duration. The three constrictor responses to stimulation of the peripheral end of the right vagus were obtained from nerves which gave vasodilatation in other trials. Elim-

nation of the afferent sympathetic components of the vagus by removal of the stellate ganglion did not alter the dilator response.

TABLE 6

*Tabulated results of stimulation of nerves on the right posterior bronchial artery; totalled trials in 23 dogs*

NERVE STIMULATED	STATUS OF OTHER NERVES	VASODILA- TATION	VASOCON- STRICTION	NO EFFECT
Intact rt. vagus.....	Intact	19	5	3
Peripheral end rt. vagus.....	Intact	27	3	4
Central end rt. vagus.....	Intact	3	3	
Peripheral end rt. vagus.....	Accelerators cut	6		2
Intact lt. vagus.....	Intact	1	1	
Peripheral end lt. vagus.....	Rt. vagus cut	1		1
Intact rt. accelerator.....	Intact	2	2	
Peripheral end rt. accelerator....	Intact		9	1
Peripheral end rt. accelerator....	Rt. or both vagi cut	1	4	

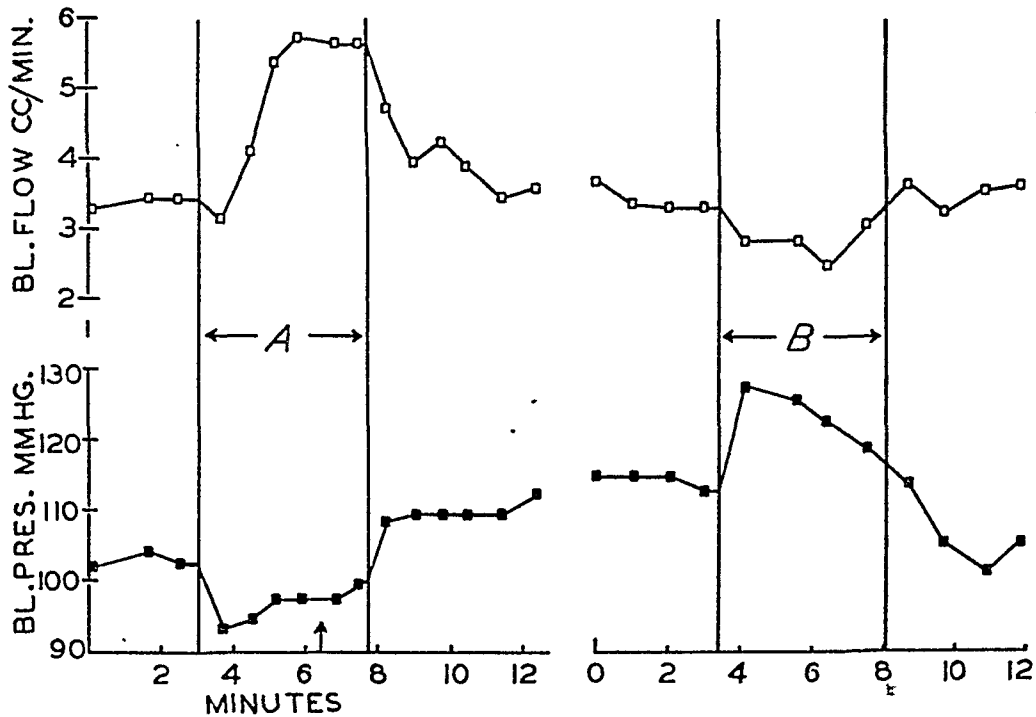


Fig. 4. Effect on right bronchial artery flow of stimulating (A) the peripheral end of the right vagus and (B) the peripheral end of the right accelerator strands. \* A and B from separate dogs with other nerves intact. Current strengths weak except at arrow in A where it was increased to moderate.

Stimulating the intact nerves or their central ends gave results which were frequently opposite in direction to those of stimulation of their peripheral end. The flows during these responses were unstable and a prolonged constrictor phase

often followed, regardless of whether dilatation or constriction had occurred. Both responses could be obtained from the same nerve but unpredictably. This variability suggests that the nerves either contain both afferent and efferent fibers with the former being able to affect the latter reflexly, or carry a mixture of both constrictor and dilator fibers, one type predominating. Only one response, the dilatation upon stimulation of the peripheral end of the accelerator fibers, can be explained by the latter alternative alone. The remaining variations and the persistent constrictor after-effect are best, or solely, explicable in terms of reflex activity.

Three additional observations point to reflex control of the bronchial circulation: *a*, Insufflation of ether or  $\text{NH}_4\text{OH}$  vapor into the isolated nostrils caused immediate vasoconstriction followed by vasodilatation; after ergotamine the con-

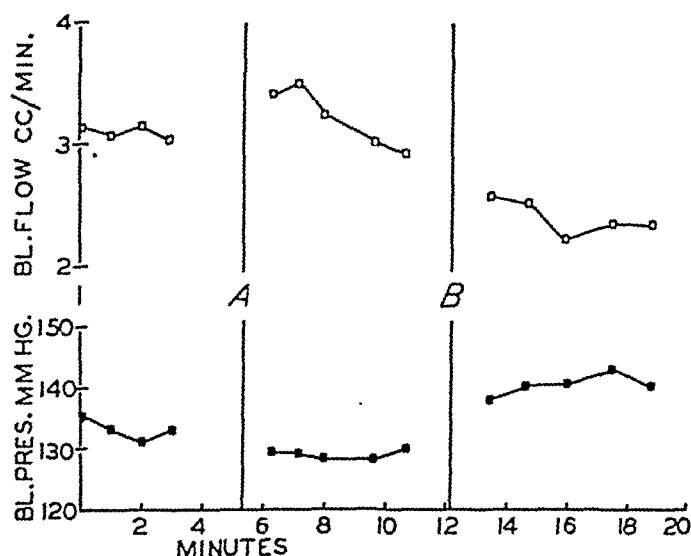


Fig. 5. Effect on right bronchial artery flow of sectioning the right accelerator branches at A and right vagus at B. All constrictor fibers probably not included in nerve section at A.

strictor phase dropped out. *b*. Variations in flow unrelated to blood pressure almost invariably occurred when the animals relaxed the bladder or anal sphincters. *c*. Deepening the anesthesia was followed by increased, decreased and unchanged flow in 30, 28 and 42 per cent of injections respectively; if the effect of these depressants were confined to the arterial or cardiac musculature, a single type of response should have been observed.

The bronchial circulation probably is under tonic vasomotor control since, in addition to certain of the data above, section of the motor nerves is followed by the predicted changes in flow. An example is seen in figure 5; in a few animals the changes were minimal while in others they were greater than that shown.

*7. Response to drugs.* Adrenalin (and U.S.P. epinephrine), ephedrine and posterior pituitary extract given intra-arterially caused vasoconstriction; systemic effects were absent unless 20 to 100 times the minimal dose was employed. The constrictor response was partially obscured by the increased flow brought about by the standard volume of saline diluent; the increase is believed the

result of lowered blood viscosity as it occurs with other liquids and plasma. This pseudodilatation prevented detection of possible vasodilatation from amounts of adrenalin less than  $0.2\text{ }\mu\text{g.}$ , the minimal dose level which gave a sharp constriction; recovery occurred in a logarithmic curve within 3 to 8 minutes. In a few trials, a dilator phase succeeded recovery but since it appeared irregularly, its origin and significance are uncertain.

Nitroglycerine, histamine, papaverine, theophylline ethylenediamine, acetylcholine and mecholyl in minimal effective doses intra-arterially caused increase of flow without systemic effects. This dilator response was differentiated from the saline pseudodilatation by the higher peak increase and the 2 to 5 minute logarithmic recovery curve; the saline increase was a simple spike less than one-third the drug increase. The minimal effective dose of the cholines, the most effective dilators, was  $0.2\text{ }\mu\text{g.}$ ;  $2.0\text{ }\mu\text{g.}$  gave a slight systemic dip in blood pressure. The artery retained its sensitivity to adrenalin and mecholyl throughout an experiment.

Intravenous administration of adrenalin and mecholyl resulted in increases and decreases of bronchial artery flow, respectively, which were synchronous with the usual blood pressure changes. Since the artery was being fed with drug-free blood from the dead space of the meter for 2 to 4 minutes after injection, unequivocal evidence of local drug effects during a systemic response was not obtained.

Adrenalin, 1:100, and histamine, 0.2 per cent, nebulized into the tracheal cannula caused decreases and increases, respectively, of bronchial artery flow without systemic effects. The decrease from adrenalin ranged between 20 and 70 per cent and both responses were persistent. This procedure offers a means of detecting bronchial absorption of drugs having a vasomotor action. The vasoconstriction from nebulized adrenalin supports the idea that "decongestion" of the bronchial mucosa is one of the special properties of this route of administering adrenalin in asthmatics. A dog sensitized to horse serum was given the shocking dose intra-arterially, but the results were ambiguous due to the severe fall in systemic blood pressure.

8. *Pharmacodynamics of the nerve supply.* The above responses to adrenalin and the cholines suggest that the constrictor fibers are adrenergic and the dilator fibers cholinergic. This was further studied as follows:

a. Ergotamine in doses of 12 to  $57\text{ }\mu\text{g./kgm.}$  intra-arterially in 4 dogs blocked completely the constrictor response to a test stimulation of the peripheral end of the accelerator fibers, while the dilator response to vagal stimulation and mecholyl was unaffected. Also it blocked the response to 10 times the  $0.2\text{ }\mu\text{g.}$  test dose of adrenalin. A delayed dilator response following adrenalin was observed twice, but since it did not consistently occur in those animals in which it was obtained, the presence of adrenergic dilator end organs is doubtful.

Ergotamine itself caused transient vasoconstriction without systemic effects in the  $12\text{ }\mu\text{g./kgm.}$  dose, whereas in the three higher doses vasodilatation preceded the constriction. The constriction in the latter instances restored bronchial flow to normal levels despite the usual hypertension.

b. Atropine, in doses of 60 to  $180\text{ }\mu\text{g./kgm.}$  intravenously or intra-arterially

in 9 dogs, effectively blocked 10 to 200 times the 0.2  $\mu$ g. test dose of acetylcholine or mecholyl; with 1000 times this dose some vasodilatation did occur. These doses of atropine, however, failed to inhibit the dilator response to stimulation of the peripheral end of the right vagus. In fact, atropine sometimes accentuated the dilatation by eliminating consensual cardioinhibition. The constrictor response to accelerator stimulation was also unmodified.

This alkaloid given intra-arterially produced a strong, persistent vasodilatation; intravenously the dilator action was slight or absent.

An effort to block the ganglionic synapses of the vagal fibers by intra-arterial nicotine failed, although a total of 2.6 mgm. was injected with marked systemic effects. A subsequent dose of atropine gave the only instance of block of the dilator response to vagal stimulation.

c. Physostigmine was given intra-arterially in doses of 0.5, 10 or 30  $\mu$ g./kgm. in 3 dogs; the two higher doses produced definite vasodilatation, the lowest none. The drug sensitized the vagal dilator fibers as judged by a lowered threshold for nerve stimulation, but because the cardioinhibitory fibers were also sensitized at the two higher doses, the vasodilatation was somewhat obscured; with the 0.5  $\mu$ g./kgm. dose, however, sensitization was apparently limited to the bronchial vessels. The response to a 0.2  $\mu$ g. test dose of mecholyl was greater after physostigmine in that the dilatation persisted longer. Aside from one dog, the peripheral accelerator fibers were unaffected by the drug. In this dog after physostigmine the bronchial flow became volatile during accelerator stimulation and when ergotamine was added a pure dilator response was obtained. This is further evidence that dilator fibers are not always restricted to the vagus nerve.

d. Cocaine, in intra-arterial doses of 0.5 or 14  $\mu$ g./kgm., appeared to lower the threshold of the constrictor response to accelerator stimulation and to increase the degree and persistency of the response to a test dose of adrenalin. These effects were not obtainable until more than 30 minutes after administration of cocaine; within this time the responses were depressed. The vagal vasodilatation was apparently unaffected by cocaine. Ergotamine superseded the cocaine effects.

To summarize, the data indicate that the vagus carries cholinergic dilator fibers and the thoracico-lumbar predominantly adrenergic constrictor fibers to the bronchial artery system. As suggested by the two exceptions mentioned, more detailed study perhaps would show that the fibers in a nerve trunk are not exclusively of one type. Evidence of cholinergic constrictors was not observed, and the occurrence of adrenergic dilators is uncertain.

9. *The effects of anoxemia and hypercapnia.* The effects of 5 or 7 per cent  $\text{CO}_2$  in 21 per cent oxygen and 10 or 7.5 per cent oxygen in nitrogen were studied in 16 trials in 3 dogs using pump and/or spontaneous ventilation. The oxygen by catheter was discontinued for these experiments without changes of flow taking place. The overall effect of all four gas mixtures was to increase the blood flow both absolute and per unit of blood pressure, although no definite pattern of response could be distinguished. The latency and after-response were variable both in extent and duration, and the flow, compared to a drug

response, was exceptionally unstable. Constrictor as well as dilator influences apparently were active. Since abnormal gas tensions are capable of affecting this circulation by reflex, and direct and indirect local mechanisms the absence of a consistent pattern would not be unexpected.

10. *The effects of increased intratracheal pressure.* In dogs ventilated by the pump, increasing the ventilation above the standard setting by reducing the air-leak always decreased the bronchial artery flow, while opening the air-leak and allowing the lungs to deflate partially always increased the flow. Since the change in inflation required to change the flow was large compared with the probable variations in the standard settings, the dispersion of the normal flows (section 2) was not due to this factor.

In dogs breathing spontaneously, positive intratracheal pressure was applied during separate and both phases of respiration by Müller valves and a mechanical device (pneumolator). In every instance pressures between 4 and 8 cm. H<sub>2</sub>O lowered the flow independently of blood pressure. In other dogs positive intratracheal pressures between 9 and 24 mm. Hg were required to stop all bronchial artery flow before blood pressure fell. These pressures overestimate the intrapulmonary bronchial capillary and venous pressure since some of the pressure was dissipated by the lung in compressing the extrapulmonary bed of the artery. These findings indicate that the pressure in the bronchial artery capillaries is not higher than that of other systemic vessels and that the pressure head is reduced in vessels proximal to the capillary in the usual manner.

DISCUSSION. The above findings indicate that the maximal flow of blood from the bronchial arteries into the pulmonary circuit is probably of the order of 1 per cent or less of the cardiac output in dogs under the conditions of these experiments. Almost exactly the same maximal flow values were obtained by use of potent vasodilator drugs as were seen during spontaneous variations in flow and hence we believe that the peak flow capacities were attained. These peak flows were at most some 5 to 6 times the average flow. It proved impossible to secure such flow values under vasodilator nerve stimulation because of concomitant cardio-inhibition. It seems unlikely that increases in bronchial artery flow of this magnitude could overtax the drainage (pressure absorbing) capacity of the pulmonary veins and raise pulmonary capillary pressure, particularly since cardiac output can be increased 5 or more times without causing pulmonary edema.

While the foregoing may reasonably be extended to man, two points make it desirable to withhold a completely negative opinion. The first is the ever present possibility of species variation: the peak bronchial flow relative to cardiac output in man might be disproportionately greater than in the dog. The second concerns the spontaneously variable flow which may result from vasodilatation of the existing bed, accrument of additional (non-collateral) bed area, arteriovenous shunts, or combinations of these; this vasodilatation may take place in either the intra- or extrapulmonary or both bed areas. Because information on these circumstances is lacking, their possible relation to pulmonary edema can be neither affirmed nor denied.

Tentatively, the spontaneous variability is regarded as a special characteristic of the bronchial artery just as other circulations such as the cerebral, coronary and pulmonary possess distinctive features. The variability is not due to the experimental set-up since it has not been observed in the coronary, cerebral, renal and femoral flows studied by this method (16, 29, 33). In its subservience to vasomotor innervation, the bronchial artery tends to be classified with the splanchnic and peripheral rather than cerebral and coronary vessels.

With few exceptions the literature on the bronchial artery has dealt with the problem of whether the bronchial artery can maintain the alveolar structure in areas whose pulmonary artery flow has been blocked or severely restricted. The data presented above suggest that the bronchial flow to a given sector of lung would be so small as to be inadequate; yet, because the alveoli are independent of blood flow for their gaseous metabolic requirements, this small flow may be ample for the remaining nutritional needs. Perhaps the respiratory movements create a gentle flux and reflux of bronchial blood in the pulmonary veins and capillaries sufficient to keep this blood in a reasonably physiologic state (34). There can be no doubt of compensatory enlargement of the bronchial arteries to lobes whose pulmonary supply has been restricted by disease or experimentally (35, 36).

Thus far no mention has been made of the reduction in oxygen saturation and tension of pulmonary vein blood brought about by admixture with the bronchial venous drainage. Calculations based on the flow data in section 4 above, assuming 98 and 60 per cent saturation of arterial and bronchial venous blood, respectively, show that the blood must have left the pulmonary capillaries at 98.4 per cent saturation with the greatest observed bronchial flow (1.02 cc./100 cc. cardiac output) and at 98.1 per cent saturation with average flow (0.28 cc./100 cc. cardiac output). The change in saturation at average bronchial flow is equal to 1.3 mm.  $pO_2$  at the 98 per cent saturation level and hence is negligible as assumed by Roughton *et al.* (37) in their analysis of the errors of the tonometric method of determining oxygen saturation; however, the difference of 0.4 per cent saturation, equal to 5.1 mm.  $pO_2$ , is of the same order as some of the errors for which corrections were suggested. It seems inadvisable at present to propose a correction for the figures commonly assigned to the arterial end of the oxygen tension gradient across the lung membranes (37, 38), chiefly because of the instability of flow and lack of data concerning the extraction of oxygen at different flows; possibly oxygen extraction varies inversely with flow, as frequently occurs in other vascular beds.

#### SUMMARY

1. In order to evaluate bronchial artery flow in the etiology of paroxysmal and other types of pulmonary edema, the volume flow through the right posterior bronchial artery was measured in 50 anesthetized dogs by the bubble-flow-meter. Techniques were described by which the flows through this single vessel can be converted to total intrapulmonary flow draining into the pulmonary veins; the factor is 1.26.

2. The flow in this vessel was highest during the initial stage of observation; it decreased with time in association with decrease of circulating blood volume, blood pressure and cardiac output. The average flow during this early stage was near 5 cc. per minute with wide dispersion of individual values; the flow appeared more closely related to cardiac output than other anatomic or physiologic factors. The average flow was near 0.3 per cent of cardiac output and the highest observed flow was 0.83 per cent.

3. A generous estimate of the peak total flow drained into the pulmonary veins is less than 1.25 per cent of the cardiac output. This is considered insufficient to embarrass the drainage capacity of the pulmonary veins and so raise pulmonary capillary pressure to the point of over-balancing osmotic forces.

4. Flow in this artery can be modified reflexly and the vessel probably is under tonic vasomotor control. It was dilated by fibers in the vagus which were apparently cholinergic and constricted by fibers in the accelerator nerve which were predominantly adrenergic. Two exceptions were observed suggesting that the accelerator nerve fibers are not exclusively constrictor in action. The artery responded to a number of drugs and abnormal oxygen and carbon dioxide tensions much like other systemic arteries.

5. The strong tendency of the flow to be cyclic or sporadically inconstant is tentatively regarded as a characteristic distinguishing it from other circulations so studied. The cause of the variations was not identified.

6. The data were discussed in relation to two other problems: the maintenance of infarcted pulmonary tissue and the magnitude of the oxygen pressure gradient across the lung membranes.

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# THE CIRCULAR COMPONENTS OF THE MUSCULARIS MUCOSAE OF THE SMALL INTESTINE OF THE DOG<sup>1</sup>

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The muscularis mucosae of the dog's small intestine contains, at all levels, both longitudinal and circular layers of muscle. In a previous report (1) the contractions of the longitudinal components were dealt with at some length. During the course of the experiments upon which this paper was based direct observation of the intestinal mucosa frequently revealed changes in the configuration of the surface which also indicated activity on part of the circular elements, but attempts to make a graphic demonstration were unsuccessful. Previous to our observations Magnus (2) and Gunn and Underhill (3) had studied circular preparations of muscularis mucosae from the cat's intestine but considered the contractions obtained too feeble to be of physiological significance.

Recently we have been successful in recording vigorous contractions of the circular submucosal muscles of the small intestine of the dog, not only in excised surviving strips, but also in the living animal with an intact circulation. A report of these experiments is herewith submitted.

**METHODS.** Excised circular strips of muscularis mucosae were prepared as follows. Sections of the small intestine 10 to 15 cm. long were removed from dogs under nembutal anesthesia and immediately flushed with and placed in cold (4°C.) physiological saline. Short segments were cut from the chilled tissue and the outer layers of muscle removed as described in an earlier communication (1). A ring, 5 to 8 mm. wide was cut from one end of the denuded cylinder, then opened, forming a strip. In the early experiments the anchoring and transmitting threads were merely pulled through the ends of the strip and tied. These preparations invariably curled and everted, leaving only the mucosal surface exposed to the saline in the bath, resulting in a rapid loss of excitability to chemical and electrical stimuli. It was found, however, that when eversion was prevented the preparations retained their irritability for hours. Eversion was avoided by looping the threads around the ends of the strip then at the moment of tying moulding the tissue so as to turn the mucosal surface inwards.

Strips thus prepared were mounted in a bath which held 200 cc. of physiological saline, the composition of which differed from Tyrode's solution in that the KCl content was a little higher (40 mgm. per cent) and that Mg was omitted. All experiments were done at 38°C. The muscle holder was constructed to support two preparations for concurrent recording and comparison. The transmitting threads were carried over a system of low-friction pulleys and attached to well balanced muscle levers with a magnification of 20 times.

<sup>1</sup> The expenses of this investigation were in part defrayed by a grant from the Bristol-Myers Company.

The experiments *in vivo* were done on nine dogs under sodium-barbital anesthesia. Short identified loops of small intestine were delivered through a mid-line abdominal incision and the outer layers of muscle freed from the submucosa as completely as possible. This was accomplished by making a longitudinal incision through the serosa and outer muscles opposite the mesenteric border for a distance of from 6 to 8 cm. then loosening the outer layers on both sides down to the point of entrance of the mesenteric vessels. It was found impossible to remove completely the band of outer muscles along the mesenteric border without seriously interfering with the circulation.

The denuded segment was lifted up until it was free from contact with the abdominal wall and held rigidly in place by clamps. On one side the wall of the segment and the free edge of the outer layers were anchored to side rods by means

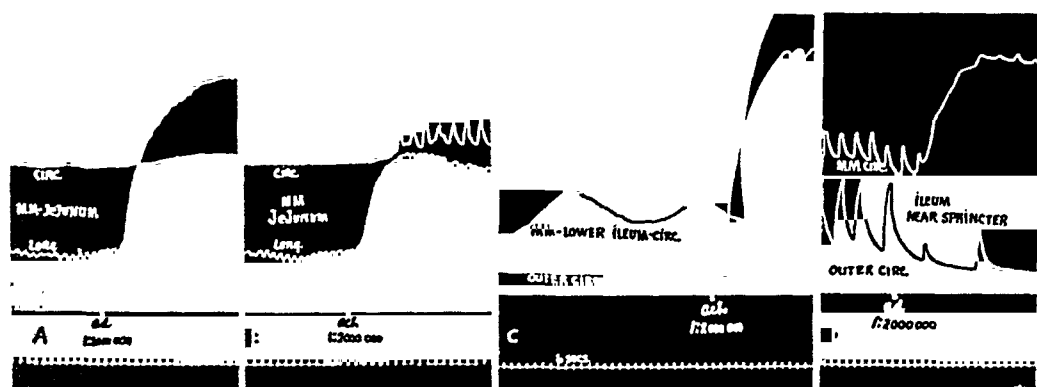


Fig. 1. The effects of epinephrine and acetylcholine on excised preparations of the muscularis mucosae of the jejunum and ileum, and on the outer circular muscles of the ileum. A, epinephrine on the circular (top) and the longitudinal (bottom) components of the muscularis mucosae of the jejunum, and B, acetylcholine on the same preparations. C, acetylcholine on a circular strip of muscularis mucosae (top) and a strip of outer circular muscle (bottom) from the ileum about 10 cm. above the ileocolic sphincter. D, epinephrine on strips of muscularis mucosae (top) and outer circular muscle (bottom) from the ileum adjacent to the sphincter.

of threads. The transmitting threads were attached to the opposite side wall and edge and carried over a system of pulleys to the recording levers. The exposed surface of the exteriorized segment was continuously irrigated with warm saline.

*Spontaneous contractions of excised circular strips of muscularis mucosae.* Preparations from all levels of the small intestine excepting the upper duodenum at times contracted spontaneously, the incidence being greatest in strips from the lower ileum. The predominant feature was a slow shortening and lengthening often requiring several minutes for the completion of a cycle (fig. 1, C). More rapid contractions, occurring at a rate of 3 to 4 per minute, were occasionally superimposed on the slower one and again the record revealed only the more rapid rhythm (fig. 1, D). This correlates well with the picture presented when observing the changes in the topography of the mucosal surface in the living animal where one sees a slow swaying of the surface involving a considerable area,

sometimes with grooves and ridges forming then disappearing within its confines, and again only small pittings appear within a sharply defined area.

*Electrical stimulation of excised strips.* Electrical stimuli were applied in a series of experiments to determine how extensively the excitatory process is conducted through the circular submucosal muscle layer. Previous observations on the effects of sharply localized mechanical stimuli applied to the mucosa in the living animal indicated that there is no widespread conduction of the contractile process, and furthermore that the size of the area involved is a function of the intensity of the stimulus (4, 5).

For the application of electrical stimuli one end of a 24 gauge platinum wire was fastened to the free end of the strip, substituting it for the transmitting thread, and the other end hooked to the power arm of the metal recording lever. This constituted the stimulating electrode. The indifferent electrode consisted of a small square of heavy platinum foil to which was welded a platinum wire long enough to extend several inches above the surface of the bath with the foil at the level of the anchored end of the muscle strip. A potential divider was used to grade the current and the circuit through the strip was completed by clipping one wire to the metal shank of the muscle lever and the other to the end of the wire supporting the indifferent electrode, with a time switch in the circuit to control the duration of the stimulus. A 60 cycle sine wave current was used.

Fluid was withdrawn from the bath, leaving only enough of the lower end of the strip immersed to insure good conduction. Graded contractions were obtained in every test on eleven different strips in response to progressively increased intensities of stimulation, until a maximal shortening was attained. In no instance were we able, using stimuli of or slightly above threshold value, to induce contractions involving all or even a major portion of the preparation. Thus there is obviously no mechanism in excised circular strips of muscularis mucosae which can mediate widespread conduction.

*Acetylcholine on excised strips.* Acetylcholine caused only excitatory responses. Many of the preparations which had been removed from the animal only a few hours reacted to dilutions of 1:500,000,000. A concentration of 1:2,000,000 usually induced maximal contractions of strips not more than 24 hours old and this dosage was routinely used, but with older preparations higher concentrations were required, and failure to react to 1:400,000 was taken as an indication of deterioration sufficient to warrant discarding the strip. The reaction to acetylcholine consisted chiefly of a prolonged tonic contraction, although in many instances rhythmical movements set in at about the time the maximal height was reached (fig. 1, B). In a few instances in which spontaneous movements were occurring, concentrations as low as 1:100,000,000 slightly augmented the height and accelerated the rate of the contractions with little or no change in the base line.

The excitatory effect of acetylcholine was not modified by treatment of the strips with nicotine, but atropine completely prevented or abolished it.

*Epinephrine.* The effects of epinephrine on circular submucosal strips were

qualitatively like those induced by acetylcholine. Its effects were not modified by previous treatment with nicotine or atropine but were abolished by ergotamine. No inhibitory reaction was encountered in any instance. The same range of dosages was employed as with acetylcholine.

In a previous communication (1) dealing exclusively with the longitudinal components of the muscularis mucosae it was reported that the maximal contractions induced by epinephrine were nearly always of greater magnitude than those caused by acetylcholine. The effects of these drugs on the circular components were different from those on longitudinal preparations in that the maximal contractions induced by acetylcholine were in most instances greater. This difference was apparent in strips from the duodenum, was more pronounced in the jejunum, and most constant and striking in the lower ileum (fig. 1, A and B).

*Vagal stimulation and acetylcholine intravenously.* Vagal stimulation induced only contractile reactions in the circular layer of the muscularis mucosae with the circulation intact, but the magnitude of the contractions was much smaller than was anticipated on the basis of the changes seen at times in the topography of the mucosal surface of the intact gut upon vagal stimulation.

The weak reactions incident to stimulation of the vagi are explained on the basis of the interruption of the majority of the peripheral vagal pathways on removal of the outer muscle layers. According to histological descriptions many small fibers pass from Auerbach's plexus to the submucosal muscular layers. This plexus is usually considered to constitute the greater portion of the terminal vagal mechanism in the gut. It is therefore not surprising that stimulation of the vagal trunks induced only small contractions.

The effects of acetylcholine in doses ranging from 10 to 25 mgm. administered intravenously (femoral vein) were inconstant, sometimes causing no change, at other times inducing small contractions. In a number of instances the denuded segment was irrigated with a 1:100,000 dilution of the drug and powerful contractions invariably occurred. Two animals were given eserine (0.1 mgm. per kilo intravenously) previous to the injection of acetylcholine. In both, the duration of the contractile period following the injection of the latter drug was considerably prolonged, and in one the magnitude of the contraction was more than doubled. On the basis of the enhancement by eserine, of the results incident to the direct application of acetylcholine to the denuded segment, and of its constant and powerful effects on excised strips, we believe that the relatively feeble reactions obtained following intravenous administration of the drug were due to its failure to reach the contractile tissues in sufficient concentration to induce maximal reactions.

*Splanchnic stimulation, and epinephrine administered intravenously.* Stimulation of the left major splanchnic nerve in nearly every instance caused contraction of the circular submucosal muscles, the magnitude often being of the same order as that obtained from surviving strips incident to the introduction of epinephrine. The effects of these contractions were plainly visible and consisted in a decrease of the diameter of the segment and the formation of grooves running chiefly in the transverse direction.

While the effect of splanchnic stimulation on the outer muscles of the gut is generally stated to be predominantly inhibitory, there are numerous published statements that this procedure may cause contraction, Bunch (6) for example. We have often observed and recorded contractile effects of splanchnic stimulation on segments of the small intestine before the outer muscle layers were removed. The explanation of this behavior most frequently advanced is that the splanchnics carry both a motor and an inhibitory innervation to the outer muscles of the gut. The possibility that this type of reaction may be due to contractions of the muscularis mucosae has not been given due consideration.

The results of experiments on intact and denuded segments of the gut (figs. 2 and 3) support the conclusion that contractions of the small intestine of the dog induced by splanchnic stimulation and by epinephrine are mediated through the submucosal musculature. Figure 2 consists of a series of records taken from a

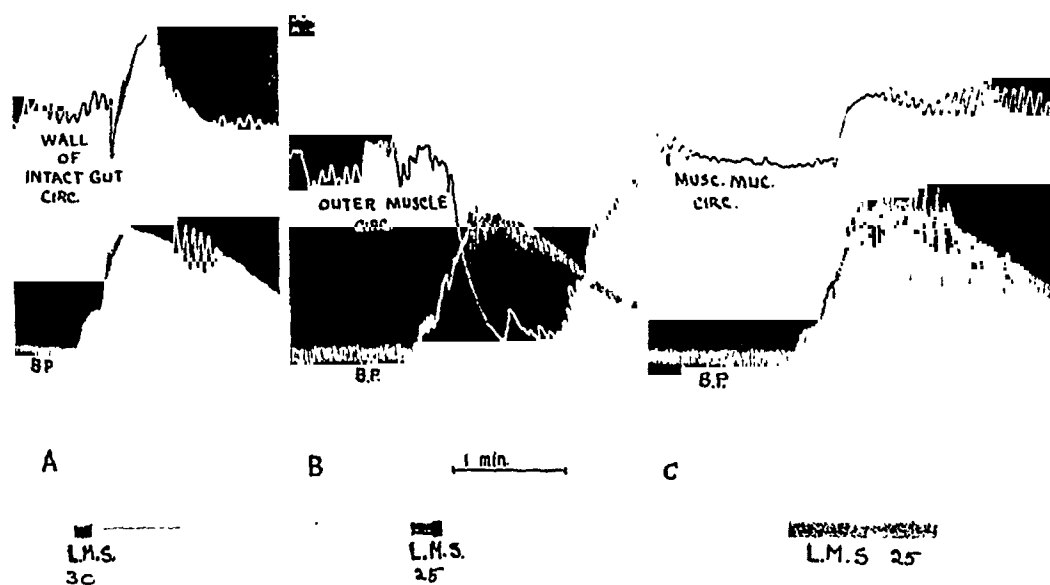


Fig. 2. Successive records from the same animal showing the effect of stimulation of the left major splanchnic nerve on the contraction of the intact gut (ileum) wall (A), the outer circular muscles (B), and on the submucosal musculature (C). Bottom record, arterial pressure.

given level of the ileum in the same animal. Portion A taken from the sidewall of the intact gut illustrates the type of reaction to splanchnic stimulation (initial relaxation followed by contraction) usually explained on the basis of a dual sympathetic innervation of the outer musculature. To obtain the record in section B the transmitting thread was attached to the edge of the freed outer muscle layers. There is no evidence of contraction until about 30 seconds after the end of stimulation. In C the recording lever was attached to the sidewall of the denuded segment. The record shows only a contractile reaction, an increase in tone upon which are superimposed strong rhythmical contractions. Figure 3 shows the effect of left major splanchnic stimulation (A) and of epinephrine (B) on the circular components of the muscularis mucosae and the outer circular muscles of the ileum recorded simultaneously. The powerful spon-

taneous contractions of the outer musculature are reflected on the record from the muscularis mucosae but the effects of splanchnic stimulation and of epinephrine on the two structures are clearly shown. The small temporary rise in the base line of the record from the outer muscles in part A during splanchnic stimulation is interpreted as being due to pull of the muscularis mucosae on the outer muscles along the mesenteric border. In part B the strong contractions of the outer muscle just preceding the relaxation following the administration of epinephrine were due to a spontaneous outburst of activity which was well under way before the drug had time to reach the intestine.

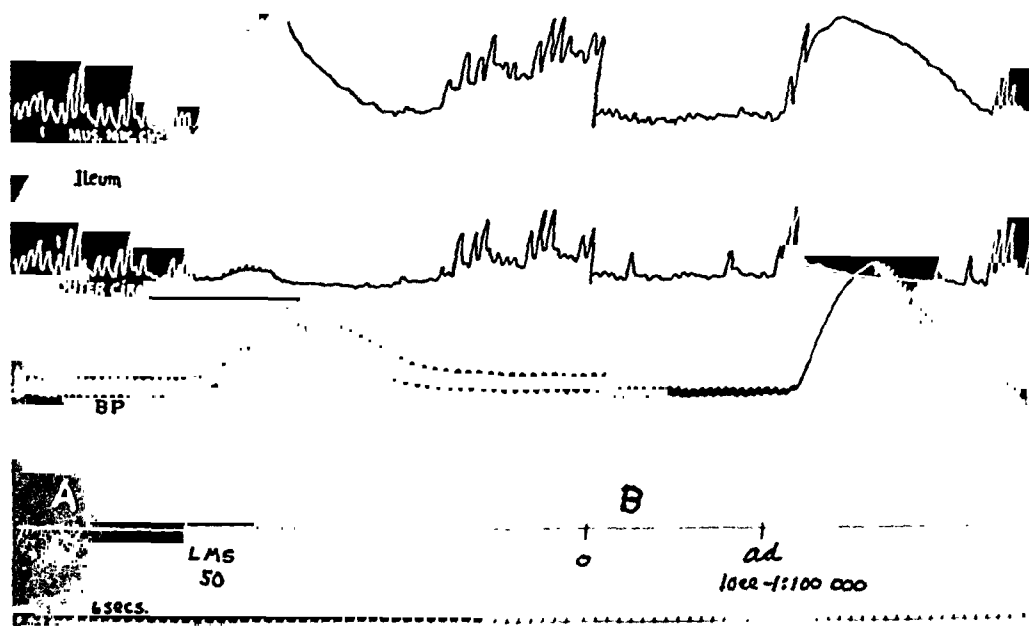


Fig. 3. Two consecutive records showing the reactions of the circular components of the muscularis mucosae of the ileum and the outer circular muscles from the same level recorded simultaneously. A shows the effect of stimulation of the left major splanchnic nerve, and B the effect of epinephrine.

The outcome of experiments with excised surviving strips of outer circular muscle from all levels of the small intestine of the dog also supports the conclusion that the only muscular structure in the gut of that animal which contracts vigorously upon sympathetic stimulation, using the effects of epinephrine as the criterion, is the muscularis mucosae. In the majority of tests the introduction of epinephrine into the bath was followed by relaxation such as is illustrated in part D of figure 1, in other instances there was no change in length, presumably because the strip was initially toneless, but occasionally small contractions were induced. A lever magnification of 20 times was used and the height of the recorded contractions seldom exceeded 10 to 12 mm. representing an actual shortening of the strip of less than one millimeter. Whether or not these small contractions reflect the activity of a sprinkling of outer circular muscle fibers with an adrenergic motor innervation, they cannot account for the often observed vigorous contractions of the gut wall induced by sympathetic stimulation.

DISCUSSION. The results of experiments described in the foregoing sections demonstrate that the circular components of the muscularis mucosae of the dog's small intestine possess contractile powers sufficient to be of physiological significance. The difficulties experienced by us in preliminary experiments, and the inability on the part of earlier investigators to induce contractions of this muscular layer were doubtless due primarily to the failure to prevent eversion and therefore to maintain an adequate  $O_2$  tension in the immediate environment of the tissues under study.

The terminal innervation of the circular components of the muscularis mucosae of the dog's small intestine is qualitatively like that described earlier for its longitudinal constituents (1). Acetylcholine, epinephrine, vagal and splanchnic stimulation induce contractions, and no evidence has been obtained for a peripheral neural inhibitory mechanism. The chief differences between the two submucosal layers lie in their relative contractile powers in different parts of the gut, and in the partition between cholinergic and adrenergic units. There is no striking or constant difference between the magnitude of maximal contractions of the longitudinal musculature induced by a given stimulus in preparations from different levels of the gut, but the maximal contractions of the circular components are much greater in the ileum than in the upper portions of the small intestine. This correlates well with the histological findings with reference to the relative thickness and distribution of the two layers. The second difference lies in the greater proportion of cholinergic units in the circular layer in contrast to the dominance of adrenergic units in the longitudinal sheet. The significance of this partition might be more evident if the embryological development of the two layers were better known.

A number of investigators (7, 8) have demonstrated a stimulating effect of a variety of chemical substances on the villi, among them being yeast extract, alanine, leucine, glucose and many of the condiments ordinarily used in cooking. Also Kokas and Ludanay (9) were able to induce vigorous movements of the villi in the dog by intravenous injection of an acid extract of the duodenal mucosa, from which they concluded that stimulation in this instance was due to a humoral substance which they named villikinin. The senior author has been able to corroborate many of these findings with respect to the villi, but at present it is not possible to state to what extent the whole submucosal musculature is involved.

In addition to the possibility that chemical substances in the chyme and humoral agents may serve as excitants to large portions of the muscularis mucosae, it is also probable that this mechanism is thrown into activity reflexly. The effects of vagal and splanchnic stimulation support this view. At present very little is known about this aspect of the problem, the solution of which will be greatly facilitated if methods for the quantitative study of the activities of the muscularis mucosae can be devised which will not necessitate the disruption of nervous pathways and the disturbance of normal mechanical relationships.

#### SUMMARY AND CONCLUSIONS

1. Methods are described by which the contractions of the circular components



of the muscularis mucosae of the dog's small intestine can be recorded, both in excised surviving strips, and in the living animal with the circulation intact.

2. The circular submucosal muscles possess both an adrenergic and a cholinergic motor innervation. No evidence has been obtained for the presence of an inhibitory innervation.

3. The contractions, both spontaneous and induced, are less pronounced and constant in the upper levels of the small intestine than in the ileum.

4. Contractions induced by local stimulation are graded in accordance with the intensity of the stimuli.

5. Evidence is presented indicating that the often observed contractile effect of splanchnic stimulation on the small intestine of the dog is due to activity of the muscularis mucosae and not of the outer musculature.

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# CHOLINESTERASES IN RAT TISSUES AND THE SITE OF SERUM NON-SPECIFIC CHOLINESTERASE PRODUCTION<sup>1</sup>

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Two recent papers (1, 2) have demonstrated that the amount of non-specific cholinesterase in rat serum is controlled at least in part by sex hormones. Estrogen elevates the serum enzyme level and testosterone depresses it, while progesterone exerts no noticeable effect except indirectly through estrogen. These conditions lead to a marked sex difference in the serum cholinesterase levels of mature rats, the female serum having several times as much enzyme as the male. Generalized damage to females, resulting from a variety of surgical procedures, depresses the serum esterase content independently of the estrogen level.

The sex hormones and other factors controlling the serum esterase levels must produce their effects by somehow altering the synthesis or destruction of the enzyme. Neither of the earlier papers, however, considered the source of the esterase. The present study, in an attempt to locate this site of serum enzyme synthesis, measures the cholinesterases in various organs and tissues of both sexes. Birkhäuser and Zeller (3) showed that the sex difference in total acetylcholine hydrolysis, which they discovered in the rat liver, does not hold in the rat brain. The liver enzyme is now known to be predominantly non-specific cholinesterase whereas the brain esterase is almost exclusively the specific enzyme. The questions therefore arise as to whether tissues other than liver and blood possess the sex difference in non-specific cholinesterase content, and whether the condition in liver is the result of synthesis of the enzyme *in situ* or simply of its storage after being produced elsewhere in the body.

Recent evidence indicates that serum cholinesterase, whether or not it is produced elsewhere, is probably synthesized by the liver: serum cholinesterase is low in liver diseases (4, 5) and it is reduced by liver injury—carbon tetrachloride administration (6); the enzyme is associated with the albumin fraction of serum proteins (7) and serum albumins are produced most readily by the liver (8). Both serum enzyme (2) and serum albumins (9) are depressed by generalized injury, and both are elevated by administering estrogen (2, 10).

The evidence adduced in the present paper corroborates and emphasizes the rôle of the liver in the production of serum non-specific cholinesterase. As a result of the study of the enzyme's distribution in various organs, a few suggestions of its possible functional rôle have been advanced. As far as we know the present study is the only published survey of the amounts of specific and non-specific cholinesterases contained in the various organs of a single species though Glick et al. (11) measured the total acetylcholine-hydrolyzing capacities

<sup>1</sup> Supported in part by a grant from the Research Council of Duke University.

of various tissues in swine. In that form Glick found total cholinesterase activity highly concentrated in salivary and lacrimal glands, fallopian tubes, alimentary mucosa, and medulla oblongata, but these earlier data of course furnish no information as to whether specific or non-specific cholinesterase, or both, are responsible for the acetylcholine-hydrolysis in each tissue.

**MATERIALS AND METHODS.** The rats employed in the present studies were all members of the inbred normal (Vanderbilt) strain. Techniques of bleeding, assaying serum cholinesterases, administering estrogens, taking vaginal smears and removing ovaries have been reported previously (1, 2). Partial hepatectomies were performed by the method outlined by Higgins and Anderson (12).

Methods of killing the animals, dissecting out the tissues, measuring their cholinesterases, and expressing results were the same as those employed by Sawyer and Hollinshead (13), utilizing the technique of Mendel, Mundell and Rudney (14) to differentiate specific and non-specific cholinesterases.

**RESULTS.** *Cholinesterases in rat tissues and organs.* The cholinesterase concentrations in various organs of each sex are summarized in figure 1. Acetylcholine-hydrolyzing capacity of each tissue is given in relative terms, the units of which make the female serum value equal to 100. In order that comparisons may be made of the amounts of specific and non-specific cholinesterase present in each tissue, the hydrolyses of mecholyl and benzoylcholine, respectively, are also included. Acetylcholine cleavage is almost invariably greater than that of either of the other two substrates. The numerous determinations of acetylcholine hydrolysis in skeletal muscle and peripheral nerve were made in a previous study (15) before specific and non-specific enzymes were recognized. The relative mecholyl and benzoylcholine hydrolyses in these two tissues represent results on only one animal of either sex.

The tissues with the highest absolute acetylcholine-hydrolyzing capacity include certain glands, the brain-stem, the spleen, red bone-marrow, and brown fat—the hibernating gland (16). In female serum, brown fat and the enzyme-rich glands except the hypophysis, the relatively high benzoylcholine values identify non-specific cholinesterase as the enzyme largely responsible for acetylcholine hydrolysis in these tissues. In the brain-stem, peripheral nerve, skeletal muscle, red marrow, spleen, lymph node, thymus, adrenal cortex and male serum, specific cholinesterase (mecholyl cleavage) is the principal enzyme hydrolyzing acetylcholine.

In most of the tissues assayed the female representative hydrolyzed only as much as, or actually less than, the male counterpart. The only tissues of considerable bulk and high enzyme concentration whose female/male ratios exceed 1, and in which, therefore, the serum enzymes might be synthesized, are gonads, liver, and brown fat. With reference to the last tissue, a comparison by statistical methods of the respective means of acetylcholine hydrolysis by male and female brown fat reveals a difference of doubtful significance ( $P > 0.02$ ). A similar comparison of non-specific cholinesterase (benzoylcholine hydrolysis) in brown fats of the two sexes shows a negligible sex difference ( $P > 0.2$ ). Therefore it is extremely unlikely that the sex difference in serum enzyme is due to a release of esterase by brown fat to serum.

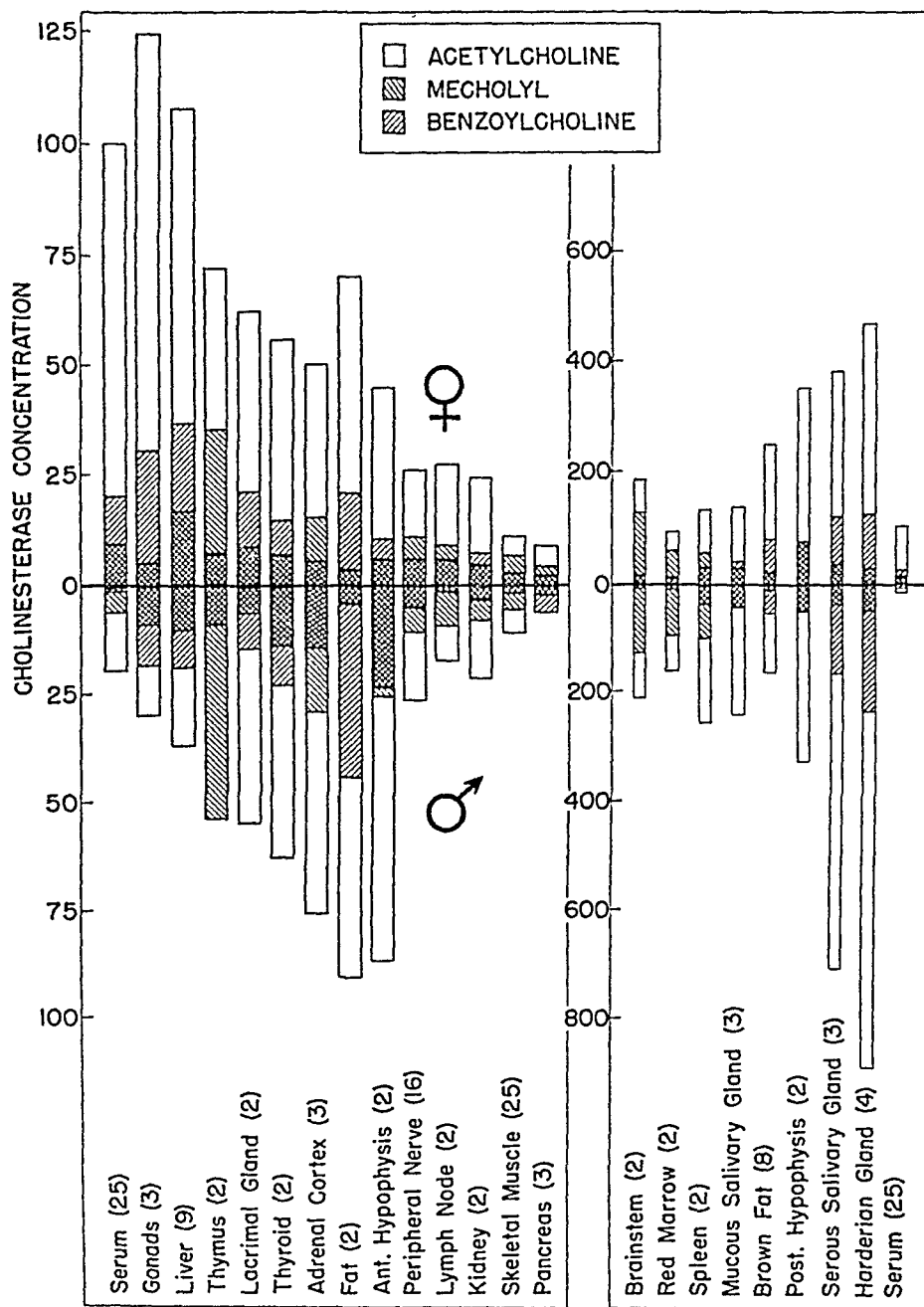


Fig. 1. Relative cholinesterase concentrations in various tissues and organs of male and female rats. Total acetylcholine hydrolysis by female serum is arbitrarily considered 100. Hydrolysis of mecholyl (specific cholinesterase) and benzoylcholine (non-specific cholinesterase) are also shown; the graphs of the three substrates overlap each other, each starting at 0. The figure in parenthesis after each tissue refers to the total number of rats from which that tissue was assayed, approximately the same number of animals of each sex being employed except in liver and serum (see table 1). It is apparent that sex differentials in favor of the female exist in the non-specific cholinesterase concentrations in serum, gonads and liver.

The difference in enzyme content of the male and female gonads is greater than in any other tissue assayed. A marked sex difference also is encountered in the accessory sex organs: the enzyme (largely non-specific cholinesterase in each

organ) in the uterus is about 3 times as concentrated as in the seminal vesicle. These are omitted from figure 1 since they are not strictly homologous. However, neither gonads nor accessory sex organs need be present to maintain a high serum enzyme content: castrate animals of either sex respond to estrogen treatment by an elevation in serum enzyme level (2) and complete hysterectomy does not modify this effect in females (17).

The liver is the only other organ of any great size whose sex differential in enzyme content favors the female. The differences between the means of the two sexes for both acetylcholine and benzoylcholine hydrolyses are highly significant ( $P < 0.001$  in each case). These differences might be the result either of enzyme synthesis by the liver or of storage of enzyme produced elsewhere and extracted from the circulating serum by liver cells. Emphasis should be placed on the fact that no other likely site of esterase synthesis was uncovered in the survey summarized in figure 1. The slight sex difference in liver specific cholinesterase content (methylcholine hydrolysis) is not statistically significant ( $P > 0.1$ ).

*Cholinesterases in rat liver and serum under various conditions.* In an attempt to ascertain whether the liver-serum enzyme relationship was a result of liver synthesis or of storage, a study was made of the relationship between liver and serum esterases under both normal and unusual conditions. The results are summarized in table 1.

In normal mature animals of both sexes the liver cholinesterase coefficients for each substrate are higher than the respective figures for serum. The most striking contrast between liver and serum values is seen in the male " $Q_{\text{Benz}}$ " figures. If the liver value were to represent storage of enzyme extracted from serum, a mechanism capable of concentrating the serum esterase more than 9 times would be necessary whereas in the female a two-fold concentration would be more than sufficient. A simpler explanation, suggested by the fact that differences between the liver and serum " $Q_{\text{Benz}}$ " values for male and female are practically equal, is that there is a liver threshold for non-specific cholinesterase of the order of 0.25-0.30 (in  $Q_{\text{Benz}}$  units). Above that threshold an equilibrium would exist between enzyme in liver and enzyme in serum, i.e., the liver-synthesized enzyme would be shared with the serum.

In accord with this proposed explanation is the fact that in young females and the first group of castrated estrogen-treated females (table 1) the serum cholinesterase levels are generally in the range expected were such a threshold acting. Liver and serum enzymes are both low in immature females and both are elevated in estrogen treated animals. The second group of castrates was unusual in that the animals maintained a low serum enzyme level for months in spite of continued estrogen administration. These were descendants of female rat 2472.1 (2, fig. 4) a line with a defective breeding record. It is significant that only the serum enzyme level is abnormal; the liver esterase values are, as is usual on estrogen treatment, elevated above the values for untreated females. The simplest explanation of this unusual liver-serum enzyme relationship would appear to be that the enzyme threshold in the liver is raised. It would seem less likely that extraction of enzyme by the liver from an abnormally low serum esterase com-

plement would result in the normal liver enzyme level. Furthermore it should be pointed out that the converse of this condition was never encountered: no animals were found with an elevated serum enzyme and a depressed liver-esterase level.

It is known that ovariectomy leads to a lowered cholinesterase level in liver (3) and serum (2). The depression, at least in serum esterase, is partially the result of estrogen withdrawal and partially the effect of generalized injury: hysterectomy, laparotomy, and castration in the presence of injected estrogen

TABLE 1  
*Cholinesterases in rat livers and sera under normal and experimental conditions*

SEX AND CONDITIONS	ENZYME SOURCE	NUMBER OF ANIMALS	QChE				
			QAch	QMech	% of Ach	QBenz	% of Ach
Male, normal, mature	liver serum	6	0.635 $\pm$ 0.080	0.182 $\pm$ 0.044	29	0.339 $\pm$ 0.021	53
		7	0.338 $\pm$ 0.011	0.114 $\pm$ 0.008	34	0.036 $\pm$ 0.004	11
Female, normal, mature	liver serum	3	1.823 $\pm$ 0.196	0.296 $\pm$ 0.044	16	0.596 $\pm$ 0.021	33
		18	1.690 $\pm$ 0.064	0.130 $\pm$ 0.006	8	0.335 $\pm$ 0.015	20
Female, young	liver serum	1	0.825 0.684	0.261	32	0.271	33
	liver serum	1	0.977 1.36	0.159	16	0.349	36
Female, castrated, estrogen-treated "high serum ChE"	liver serum	1	2.66 2.87	0.313	12	0.754	28
	liver serum	1	3.01 2.26	0.481	16	0.906	30
Female, castrated, estrogen-treated "low serum ChE"	liver serum	1	2.49 0.95	0.352	14	0.888	36
	liver serum	1	1.93 1.15	0.337 0.148	17 13	0.639 0.210	33 18

all induce temporary depressions of serum cholinesterase content. Since it seems likely that these depressions are not due to the production of inhibitors of esterase activity but that they represent an actually diminished amount of circulating enzyme (2), the depressions probably reflect either an interrupted synthesis of the enzyme or an accelerated destruction.

A preliminary partial hepatectomy experiment, conducted prior to the discovery of the effects of generalized injury, revealed a liver enzyme content within the normal range at a time when the serum esterase still appeared to be somewhat depressed (18). In view of the subsequent findings of the effects of generalized injury it seemed especially desirable to repeat the partial hepatectomy experi-

ment under more carefully controlled (castrates injected with estrogen) conditions. The experiment, summarized in figure 2, employed 3 estrogen-treated castrates. Their serum enzyme levels were followed before and after castration, and, when they had recovered from the effects of that operation, 2 of the 3 were partially hepatectomized. In the 2 experimental animals the serum enzyme was depressed to practically the same extent as after castration, again indicating an effect of generalized injury. At autopsy 3 days after the second operation the enzyme concentrations in the liver remnants of the experimental animals were found to be indeed depressed, the rat with the lower serum enzyme level revealing the lower liver enzyme concentration. The altered enzyme level in liver con-

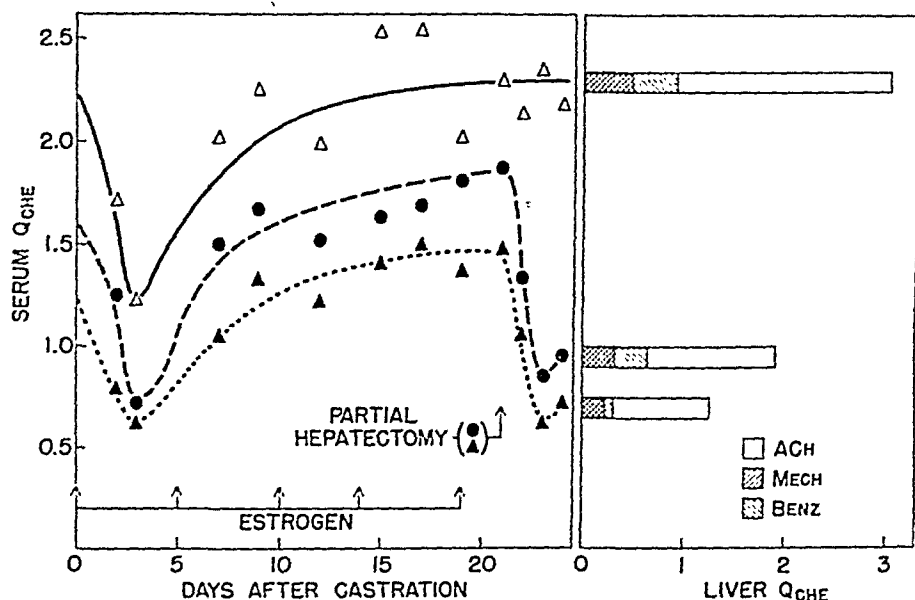


Fig. 2. Effect of partial hepatectomy on the cholinesterase concentrations in sera and in the respective liver remnants. The effect of castration (in the presence of estrogen) on serum enzyme levels is also shown for comparison. Estradiol benzoate (50  $\mu$ g.) was injected subcutaneously into each of the animals on the days indicated by the arrows.

tributes no information concerning the liver synthesis-versus-storage problem. However, assuming from the earlier evidence that serum non-specific cholinesterase is synthesized in the liver, the conclusion may be reached that generalized injury depresses serum enzyme content by temporarily blocking cholinesterase synthesis in the liver rather than by increasing its rate of destruction.

**DISCUSSION.** Many of the results have been discussed as they were presented. A consideration of the function of the non-specific enzyme and a summary of the evidence for its synthesis by liver remain to be treated.

Specific cholinesterase is concentrated in nerve fibers and endings but is also found in large amounts in red blood cells and sera and glands of certain species (19, 20, 14). In the present study it is probably associated with developing erythrocytes in red bone-marrow, but its presence in the perfused lymph node, spleen and thymus would indicate that the lymphocyte may also contain specific

cholinesterase. Its function there, as also in the erythrocyte, is obscure. By far the highest concentration of specific enzyme is found in the brain-stem, in keeping with the acetylcholine-cholinesterase theory of neural transmission (21).

Non-specific cholinesterase is apparently never highly concentrated in nerve fibers and endings (22, 23). Removal of the cholinergic nerve endings from an organ rich in non-specific cholinesterase (cat superior cervical sympathetic ganglion) has relatively little effect on the content of the enzyme (13). However many of the organs found in the present study to be rich in non-specific cholinesterase are known to be sensitive to acetylcholine or to utilize a cholinergic mechanism of neurochemical transmission. For instance, intense salivation is induced in the rat by injecting either acetylcholine or eserine, a potent anti-cholinesterase. Secretion by the lacrimal and especially the Harderian glands is so sensitive to acetylcholine administration that the latter is used in a biological assay method for the drug (24). The response is the production of "bloody" tears (chromodacryorrhea). Secretion of at least one hormone by the rat anterior pituitary gland is said to be induced by the administration of acetylcholine (25). It is not unlikely that in the other glands the high concentration of non-specific cholinesterase is also associated with a cholinergic mechanism of the induction of secretion. Surprisingly enough the pancreas, which in the guinea pig is especially rich in the enzyme, has practically no cholinesterase of either kind in the rat.

It is possible also that in such sites as brown fat, ordinary fat, and ovary, non-specific cholinesterase is associated with an acetylcholine-mediation mechanism. However in these sites an alternative explanation seems more probable. It is known that the enzyme also hydrolyzes such simple esters as tributyrin and methyl butyrate. Might not its principal function in these sites be that of a simple lipase concerned with lipid metabolism? A simple lipase (esterase) was demonstrated in brown fat by Vignes (26). Similarly Glick (27) demonstrated that in the pig stomach acetylcholine hydrolysis was strongest in the inner mucosal layer, the same distribution as he had found for methyl butyrase (28). It seems probable that Glick was measuring the cleavage of two substrates by the same enzyme, non-specific cholinesterase. Was its principal function the hydrolysis of acetylcholine or of other esters?

It should be remembered that in the rat the serum enzyme can be inhibited by triorthocresyl phosphate to a degree as great as 80 per cent without producing noticeable physiological effects (29, 1). More recently Hawkins and Gunter (30) have found that practically complete inhibition of the non-specific enzyme induces physiological symptoms in the dog only when the specific enzyme is also inhibited. Thus it would appear that the serum non-specific cholinesterase plays, at best, only an auxiliary rôle in cholinergic mechanisms.

The influence of estrogenic hormones *in vivo* on the cholinesterase content of rat liver and serum may be related to the fact that estrogens produce cholinergic effects (31). Torda (32) found that estrogens slightly depressed specific cholinesterase *in vitro* but interestingly enough estrogens stimulated acetylcholine



synthesis while androgens depressed it (33). The augmented non-specific cholinesterase content may develop secondarily to the increased ability of tissue to synthesize acetylcholine.

The present evidence indicating that serum esterase is synthesized by the liver is summarized below. Only liver and gonads display the marked sex difference in enzyme content characteristic of the serum. The gonads are not necessary to the synthesis since castrates respond to administration of estrogen by an elevation of liver and serum cholinesterases. In both sexes a relatively constant enzyme threshold in the liver, above which level the esterase is liberated into the serum, constitutes a simpler explanation of the known facts than does a differential concentrating mechanism from serum to liver. The liver produces serum albumins (8), and serum cholinesterase has been associated with the albumin fraction of serum proteins (7). In keeping with the last statement are the facts that liver damage lowers serum albumins (34) and liver and serum cholinesterases (6); liver diseases lower both serum albumins and cholinesterase in humans (5); the "alarm reaction" lowers serum albumin concentrations (9) and serum cholinesterase (2); estrogens elevate serum albumin levels (10) and serum non-specific cholinesterase (2). Further stages in the mechanism by which estrogen elevates and testosterone depresses cholinesterase synthesis by the liver will be presented in a subsequent article.

#### SUMMARY AND CONCLUSIONS

A survey of the cholinesterases in various tissues and organs of the albino rat reveals high concentrations of the non-specific cholinesterase in salivary and Harderian glands, brown fat (hibernating gland), ovary, uterus, liver and blood serum. Specific cholinesterase is the predominant esterase in the brain-stem, red bone-marrow, spleen, thymus, lymph node, adrenal cortex, skeletal muscle and peripheral nerve.

A marked sex difference in non-specific cholinesterase content, previously noted in serum (the female contains much more than the male), is also encountered in gonads and liver. Since castrates of either sex respond to the administration of estrogen by elevations of their liver and serum enzyme content, it is concluded that the liver is the site of synthesis of the serum non-specific cholinesterase.

Specific cholinesterase in nerves and effector organs is concerned with cholinergic neural transmission but its function in the red blood cell and lymphoid organs is obscure. The non-specific cholinesterase in glands is perhaps also associated with cholinergic mechanisms but in adipose tissues and serum it is more likely concerned with the hydrolysis of simple esters other than acetylcholine.

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# RENAL HYPEREMIA IN DOGS INDUCED BY ORAL ADMINISTRATION OF CINCHONA ALKALOIDS<sup>1</sup>

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The purpose of the investigations reported in this paper was to measure the effect of oral doses of the cinchona alkaloids on the renal plasma flow and glomerular filtration rate in dogs. It has long been known that large oral doses or intravenous administration of some of these alkaloids can cause a marked fall in arterial blood pressure and a peripheral vasodilatation (4, 11). This fact prompted us to study the effect on the circulation in the kidney of oral doses no larger than those used in therapy. Renal clearance methods make it possible to measure circulatory changes in the kidneys without anesthesia or surgery. Changes in the volume of the renal circulation are of obvious importance because of their far-reaching effects on the general physiology of the animal.

**PROCEDURES.** Timed urine collections by catheter and venous blood samples were obtained from trained female dogs loosely restrained and unanesthetized. During the longer experiments the dogs were released into a cage between the periods of sample-taking. The renal clearance of p-aminohippurate (hereafter called PAH) at plasma concentrations around 2.0 mgm. per cent was taken as a measure of the effective renal plasma flow (16). The renal clearance of exogenous creatinine was calculated as the equivalent of the glomerular filtration rate (13).

Proper blood levels of PAH and creatinine were maintained by constant intravenous infusion or, in longer experiments, by repeated subcutaneous injections.<sup>2</sup>

There was some variation in the manner of establishing control values. In the majority of experiments, several ten minute clearance periods were taken in the morning, after which the alkaloid was administered in the form of the sulfate in gelatine capsules. At varying intervals of time after this dose, more blood and urine samples were taken. In some experiments the control observations were obtained one day and the experimental data the following day, the dogs being on a constant diet. In several experiments samples were taken also 24 to 48 hours after the dose.

Plasma filtrates were prepared by the cadmium method of Fujita and Iwatake

<sup>1</sup> This research was supported by a grant from the Samuel S. Fels Fund.

<sup>2</sup> Since Beyer et al. (2) have reported that PAH clearances after oral doses of this agent were essentially the same as with intravenous administration we tried administering PAH and creatinine orally. We were able to establish proper plasma concentrations but the technique did not prove satisfactory because the PAH clearances after several hours fell progressively until they were little higher than the creatinine clearances. This seems to indicate that the PAH is altered, perhaps to p-aminobenzoate, and probably in the large gut.

(7). PAH concentrations in plasma and urine were determined by the method described by Smith et al. (16). Creatinine concentrations were measured by the alkaline picrate method of Folin and Wu (5). The concentration of cinchona alkaloid in the plasma was determined by the method of Brodie and Udenfriend (3).

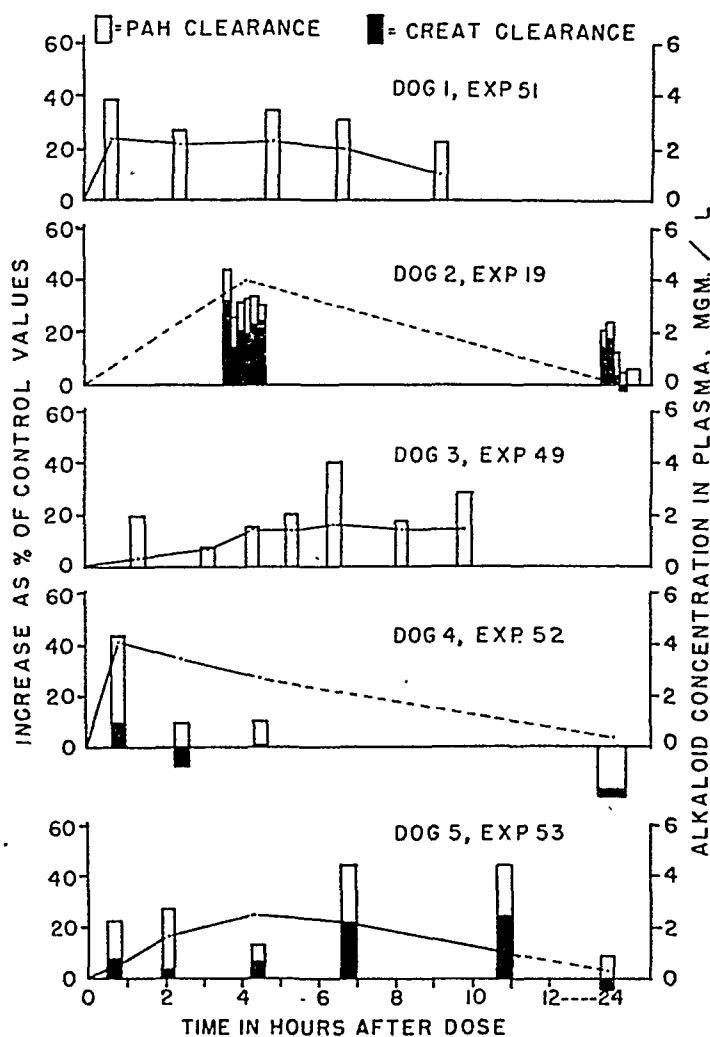


Fig. 1. The effect of 20 mgm. per kgm. of quinidine sulfate, orally, on the effective renal plasma flow (PAH clearance) of five dogs. The dose was given at 0 time. Plasma concentrations are plotted against the ordinate on the right side. The change in the glomerular filtration rate (creatinine clearance) is shown in experiments 19, 52 and 53.

Mean systemic arterial blood pressures were recorded by puncturing the femoral artery with a needle attached to a mercury manometer.

**RESULTS. A. Effective renal plasma flow.** In 14 out of 19 controlled experiments the cinchona alkaloids caused a definite increase in renal plasma flow over the control levels. In the 14 positive experiments quinidine was used in 9 instances, cinchonidine in 2, cinchonine in 2, and quinine in 1. In the 5 negative experiments quinidine was used in 3, cinchonidine in 1, and cinchonine

in 1. Quinidine was used in most of the experiments because of an early impression that it was the most effective of the cinchona alkaloids. However, we were able to establish renal hyperemia with all four alkaloids in one dog as shown in figure 2.

In two of the five negative experiments the control values were elevated, as compared with other values obtained on other occasions with the same dogs, suggesting the hyperemia accompanying febrile episodes. This was ruled out in later experiments by checking the rectal temperature before and during the experiment.

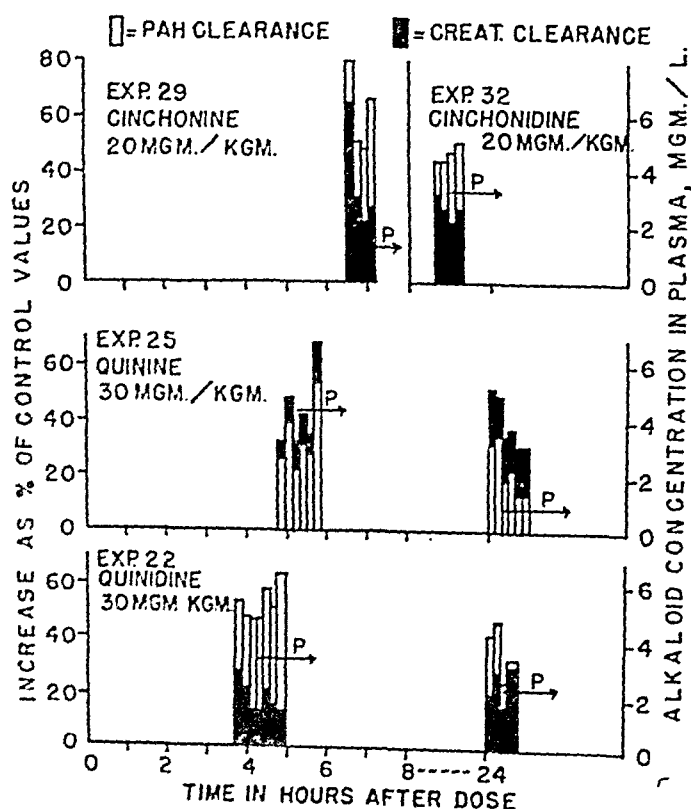


Fig. 2. The effect of oral doses of the four principal cinchona alkaloids on the effective renal plasma flow (PAH clearance) and glomerular filtration rate of dog 2. P = average plasma concentration of the alkaloid during each group of clearance periods. (Refer to the ordinate on the right.)

The time course of the effect is indicated in figure 1 where the results on five different dogs, each of which was given a 20 mgm. per kgm. dose of quinidine sulfate, are shown. The effect begins in less than half an hour and lasts for several hours according to the sojourn of the drug in the blood. In some experiments there was still some hyperemia after 24 hours. In experiment 53, shown at the bottom of figure 1, it is demonstrated that a second dose of quinidine produces and enhances the effect. The second dose was given  $2\frac{1}{2}$  hours after the first. The magnitude of the effect is influenced by the concentration of alkaloid in the plasma but does not correlate closely with it. With doses of 20 mgm. per kgm. the PAH clearance is increased by at least a third in most instances

but seldom exceeds a 50 per cent increase. This degree of renal hyperemia accompanies plasma alkaloid concentrations of 1 to 5 mgm. per liter of plasma.

B. *Filtration rate.* The glomerular filtration rate, as measured by the creatinine clearance, also increases in most instances after the administration of one of these alkaloids. With one exception (expt. 25, fig. 2) the filtration rate does not increase to the same degree as the renal plasma flow. This indicates a decrease in the intraglomerular pressure in spite of the increased perfusion of the glomerular capillaries.

C. *After effects.* With one exception (expt. 52, fig. 1), there is no evidence of a depression of the renal circulation after the hyperemic effect disappears. With the disappearance of the alkaloids from the plasma the clearances of PAH and creatinine usually return to the control levels.

D. *Blood pressure and pulse rate.* There is no marked change in the mean arterial blood pressure, with oral doses of the alkaloids of 10 to 30 mgm. per kgm. of body weight and with plasma concentrations up to 10 mgm. per liter. The pulse rate increases with the higher plasma concentrations of the alkaloids.

E. *Intravenous administration.* In four experiments quinidine sulfate was given by intravenous infusion at the rate of 1.5 to 2.5 mgm. per minute. This rate establishes plasma concentrations similar to those which follow the oral doses described above and also does not markedly depress the femoral blood pressure. This infusion did not cause much change in the renal circulation but after the infusion was stopped a marked renal hyperemia developed in two instances.

DISCUSSION. Agents which have been demonstrated previously to cause a sustained increase in the renal circulation of dogs are pyrogenic substances (9) and a high protein diet or intravenous amino acids (12). This effect of pyrogenic agents has been investigated extensively in man (14, 15). That removal of one kidney will cause an increase in the circulation of the remaining kidney has also been demonstrated in dog and man (6, 17).

In considering the mode of action of these alkaloids on renal circulation, we can exclude a rise in systemic arterial pressure leaving a choice between a neurogenic and a humoral action on the smooth muscle of the renal arterioles. There is considerable evidence to indicate that cinchona alkaloids have an action antagonistic to autonomic influences, sympathetic (4, 11) as well as parasympathetic (1, 10). On the other hand, it has been demonstrated that in the resting, unanesthetized dog or man there is no sympathetic renal vasoconstriction and there is no evidence of any vasodilator nerve supply (15, 9). This would seem to indicate that the alkaloids act directly on the smooth muscle of the renal arterioles to cause dilatation. We do not have the evidence to settle this point. It is likewise not yet possible to decide whether the increase in the pulse rate reflects a removal of the tonic vagus action on the heart due to the vagus-blocking action of these drugs (1, 9), or whether it may be a reflex compensation for the peripheral vasodilatation.

The renal vasodilatation shown in our experiments and the well known lowering of the blood pressure with large oral doses or intravenous administration of cinchona alkaloids are properties which might be of value in the therapy of certain

types of hypertension and renal disease. Similar properties prompted a clinical trial of some of the pyrogens in patients with essential hypertension but, although a lowering of the blood pressure was achieved, the investigators concluded that it was due to adverse and undesirable hemodynamic alterations (8). Our animal experiments suggest, therefore, trial studies of the possible application of the renal circulatory effects of the cinchona alkaloids to problems of human hypertension and renal insufficiency. Fortunately, several centuries of therapeutic use of these agents in man has practically excluded unexpected side effects.

#### SUMMARY

1. The effect of oral doses of the sulfates of the cinchona alkaloids on the effective renal plasma flow, as measured by p-aminohippurate clearances, and the glomerular filtration rate, as measured by creatinine clearances, has been studied in dogs.

2. These agents cause an increase in effective renal plasma flow of 30 to 50 per cent beginning within 30 minutes and lasting for several hours according to the sojourn of the drug in the blood.

3. The filtration rate is also increased but to a smaller degree.

4. There is little change in blood pressure but there is usually some acceleration of the pulse rate.

5. The manner of action and possible therapeutic applications are discussed.

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# INFLUENCE OF THE THYROID HORMONE ON THE EFFECTOR SYSTEMS OF THE MAMMALIAN HEART

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The concept of hyperthyroidism is generally associated with a state of "sympathetic hyperexcitability", whereas, in hypothyroidism, it is believed that the excitability of this system is low. The experimental evidence which supports these viewpoints is inadequate. In hyperthyroidism it is thought that the adrenergic effectors react more intensely to sympathetic impulses; this belief is based on the fact that under the influence of long continued administration of thyroid hormone some organs become more sensitive to adrenaline (2, 8, 11, 15, 18, 21). In hypothyroidism, on the contrary, there exists a lower sensitivity to this neurotransmitter substance (6, 18, 19, 20).

The influence which thyroid hormone exerts on cholinergic neuroeffector systems has scarcely been investigated. A paper by Albertoni (1), published in 1916, provides some evidence that in dogs and rabbits treated with fresh thyroid gland, the effect of vagus stimulation on the heart is less evident than in normal animals. This observation, as far as we know, has not been confirmed. Therefore it seemed of interest to study, in hyper- and hypothyroid animals, the response of the heart to vagus stimulation. At the same time we thought it necessary to learn whether either lack or excess of thyroid hormone modifies the response of the heart to the vagal transmitter substance. In connection with the action of acetylcholine (AC) on the isolated (normal) heart we should refer to the fact recently stated by McDowall (13) in England, by Haney and Lindgren (7) in the United States and by us in Chile (9, 10) that AC has, in appropriate concentrations, a stimulating effect which manifests itself with special intensity after atropinization of the heart. The analysis of the mechanism of this phenomenon revealed that the stimulating effect of AC is satisfactorily explained by the presence in the heart of structures which behave pharmacologically like sympathetic ganglia and which, under the stimulus of AC, liberate adrenaline which in turn is responsible for the cardiostimulation.

These facts seem to show that AC must act on the heart through two different mechanisms, since it exhibits both a "muscarine like" and a "nicotine like" action. If we refer this latter action of AC to the above mentioned fact, that in hyper- and hypothyroidism there exists a modification of the sensitivity of the myocardium to adrenaline, then we must consider the possibility that thyroid hormone may modify also the response of the heart to AC.

The present paper gives an account of two series of experiments performed on normal and thyroidectomized animals and on animals treated with thyroid hormone. The first series (A) refers to the response of the heart (in the whole



animal) to vagal stimulation, and the second (*B*) to the response of the isolated heart to AC.

**EXPERIMENTAL. General Technique.** Animals used in the experiments of series *A* were cats, rabbits and rats; and for those of series *B*, cats and guinea pigs.

(1) Cats and guinea pigs, thyroidectomized under ether anesthesia, were used 2 weeks to 5 months after operation. Tetany (never observed in the guinea pigs) was controlled in the cats by giving milk fortified with calcium gluconate for a few days post-operatively. In cases of tetany, 1 cc. Hytakerol (AT 10, Winthrop) was given daily by mouth.

(2) *Hyperthyroidism* was produced in the following way: (a) *Cats* (normal or previously thyroidectomized) were injected with thyroxine (0.5–1 mgm. per kgm. subcutaneously) daily during 2–8 weeks. (They were fed with raw meat and food scraps from the hospital kitchen.) Animals which lost weight rapidly and progressively could not be injected for a sufficiently long time to develop heart signs. Only those cats were used which either recovered or increased their earlier weight after an initial drop.

(b) *Rabbits* (of 2 kgm.) were given 1–2 grams of dried thyroid powder daily through a gastric tube, for 8–15 days. They were fed rabbit chow, carrots and fresh alfalfa.

(c) *Guinea pigs* (normal or previously thyroidectomized) were given thyroxine (1  $\mu$ g. per gram of body weight subcutaneously) daily for 8–15 days. A daily control of oxygen consumption was made. The guinea pigs were used for the experiment when the heat production had risen from the normal values of 35–40 up to 60–70 Cal./m<sup>2</sup>/hour. (The animals were fed in the same way as the rabbits.)

(d) *Rats* (about 220 grams) were injected with thyroxine (10  $\mu$ g. per gram daily) for 10–30 days or given thyroid powder by mouth (1 to 1.5 grams daily). The rats, kept in individual cages, were fed a commercial chow ad libitum.

(3) *Anesthesia.* (a) *Cats* (normal and thyroidectomized) were given Dial (0.6 cc. per kgm. intraperitoneally). For the hyperthyroid animals, which are very sensitive to barbiturates, 0.3 to 0.4 cc. per kgm. sufficed.

(b) *Rabbits* were anesthetized with urethane; normal animals were given 1 gram per kgm. and hyperthyroid animals 0.5 to 0.8 gram per kgm., subcutaneously.

(c) Normal and thyroidectomized *guinea pigs* and normal *rats* were injected subcutaneously with Nembutal 6 mgm./100 grams and the hyperthyroid animals with 3–4 mgm. per 100 grams.

*Series A. Heart in situ.* The peripheral end of the vagus was stimulated in the neck, with bipolar shielded silver-wire electrodes (distance of approximately 5 mm.). For the stimulation a.c. 50 cycles was used (shielded transformer, output 8 volts). The voltage was regulated by a potentiometer. For the stimulations supramaximal intensity was used. In all experiments series of 10–15 stimulations of 15 seconds' duration each, with intervals of 15 seconds, were made.

The effect of vagus stimulation on the heart was observed in cats and rabbits

by recording the blood pressure with a membrane manometer attached to the left carotid artery.

In one group of rats artificial respiration was induced, the thorax was opened and heart volume and frequency were recorded by means of a cardiometer connected to a Marey drum. In another group of rats only heart frequency was recorded without opening the thorax, by means of deriving heart action potentials with subcutaneous electrodes (one on the thorax and the other on a hind leg). The potentials were amplified with a condenser-coupled amplifier and recorded with an electromagnetic signal.

*Series B. Heart in vitro.* The hearts were isolated according to Langendorff, in the way previously described (10). AC was injected into the coronary system through a cannula inserted close to the heart, in a constant volume of 0.1 cc. The solution of this drug was always freshly prepared without adding acid. Adrenaline was given in the same way.

For the detection of the adrenaline-like substance liberated from the guinea pig heart the rectal cecum of the fowl was used as described (10), this preparation giving much more reliable quantitative results than the rabbit's intestine.

The experiments were conducted as follows: The heart of a hyperthyroid animal was isolated and perfused with Tyrode solution. The perfusate was collected, atropine was added ( $10^{-6}$ ) and this solution, "normal perfusate", was used to irrigate the rectal cecum of a chicken, which had been prepared simultaneously. At the beginning of the experiment the threshold dose of adrenaline for the heart was determined, at the same time as the sensitivity of the rectal cecum to this drug. Most of these preparations responded with a pronounced relaxation to a concentration of adrenaline of  $10^{-10}$ , but exceptionally there were intestines even more sensitive. In the course of the experiment repeated controls of the sensitivity of the cecum to adrenaline were made.

After having determined the threshold response of the heart to adrenaline, the injections of AC into the heart were started. The drug was injected in increasing doses in order to determine the threshold for the "muscarine-like" and "nicotine-like" effects of AC. (The doses were: 0.1, 0.5, 1, 5, 10, 20, 30, 50 and 100  $\mu\text{g.}$  of AC.)

For the quantitative determination of adrenaline liberated by the heart—for which purpose guinea-pig hearts are most suitable—the following procedure was used: 20  $\mu\text{g.}$  of AC were injected into the heart and at the same moment the collecting of the perfusion fluid of the heart was started till 10 cc. had been obtained; the time necessary for collecting this volume was recorded thus giving a measure of the coronary flow.

A few minutes before injecting the amount of 20  $\mu\text{g.}$  of AC into the heart, the rectal cecum was irrigated with atropinized "normal perfusate" to which AC had been added in a concentration of 2  $\mu\text{g.}$  per cc. In this way the concentration of AC of the irrigating fluid became similar to that of the heart perfusate obtained during the action of 20  $\mu\text{g.}$  of AC. The collected perfusate was atropinized before being used for the irrigation of the intestine. After the solution had acted on the intestine, this was washed with "normal perfusate".

For the determination of adrenaline liberated by the heart by 50  $\mu\text{g.}$  of AC

the same procedure was used: the rectal cecum was irrigated with "normal" atropinized perfusate containing AC in a concentration of 5  $\mu$ g. per cc. After injecting 50  $\mu$ g. of AC into the heart, the perfusate was collected again in a volume of 10 cc., atropinized, and used for the irrigation of the intestine. The adrenaline-like activity of the two perfusates was compared with known concentrations of adrenaline.

After having determined the sensitivity of the hyperthyroid heart to AC and adrenaline, and having titrated the adrenaline released from the heart by doses of 20 and 50  $\mu$ g. of AC, a heart of a thyroidectomized animal was isolated and treated in the same way, using the same rectal cecum for the titration of the adrenaline-like substance liberated by the heart during the action of 20 and 50  $\mu$ g. of AC.

At the end of the experiment atropine was added to the Tyrode solution perfusing the heart in a concentration of  $10^{-6}$ . This procedure was used because the hearts of thyroidectomized animals are, as will be described later, very sensitive to the depressor action of AC and only slightly sensitive to the stimulating effect of this drug. Atropine abolishes the first action of AC eliminating also its pronounced muscarine-like effect on the coronaries. (After atropinization the coronary flow of the guinea-pig heart diminishes with AC, so that adrenaline, if liberated at all, appears in a higher concentration in the perfusate.) The titrations of adrenaline were repeated after atropinization of the heart, using the same doses of AC (20 and 50  $\mu$ g.).

The following drugs were used:

Acetylcholine chloride, Hoffmann-LaRoche, Inc.

Eserine salicylate, May and Baker.

Prostigmine, Hoffmann-LaRoche, Inc.

Atropine sulphate

Epinephryn F.E.U. Optimo, S. B. Penick & Co.

**RESULTS OF SERIES A. Rabbits.** In 10 experiments on *normal* rabbits vagus stimulation produced the characteristic heart depression, which was maintained almost unaltered till the end of the stimulation series (10 or 15 stimulations).

From a group of 14 *hyperthyroid* rabbits 2 reacted with normal heart depression and 3 with acceleration and slight rise of blood pressure ("paradoxical effect of vagus stimulation on the heart"). In 4 cases the effect of vagus stimulation on the heart lost its intensity rapidly and was, at the end of the stimulation series, completely abolished. In 5 experiments the vagus stimulation had no effect on the heart, in spite of the fact that the stimulation was raised to six times its usual intensity. In 3 of these cases, after intravenous eserine injection (50 to 100  $\mu$ g.) there appeared a pronounced effect of the vagus stimulation on the heart. In the remaining experiments eserine had no effect.

**Cats.** Fifty-three experiments on non-treated cats were done. The hearts of these animals showed great variations in their responses to vagal stimulations. In 5 of these experiments, the effect of vagus stimulation on the heart was at the beginning of the stimulation series rather weak, disappearing completely after a few stimulations. In 11 cases the vagus stimulation yielded intense effects

on the heart at the beginning of the series, but the intensity of the heart response decreased progressively and was at the end considerably diminished. In the rest of the experiments constant and well sustained heart effects were obtained.

In 7 experiments on *thyroidectomized* cats vagal stimulation gave a striking constancy of the heart responses, which remained unchanged till the end of the stimulation series. The pulse pressure of thyroidectomized animals was remarkably low. Figure 1a shows a typical example of vagus stimulation in a thyroidectomized cat.

The experiments on 19 cats *treated with thyroxine* and 5 *previously thyroidectomized and afterwards made hyperthyroid* were characterized by the fact that the effect of the vagus stimulation lost its intensity very rapidly, yielding at the end of the stimulation series only scarcely noticeable depressions. Twelve of the

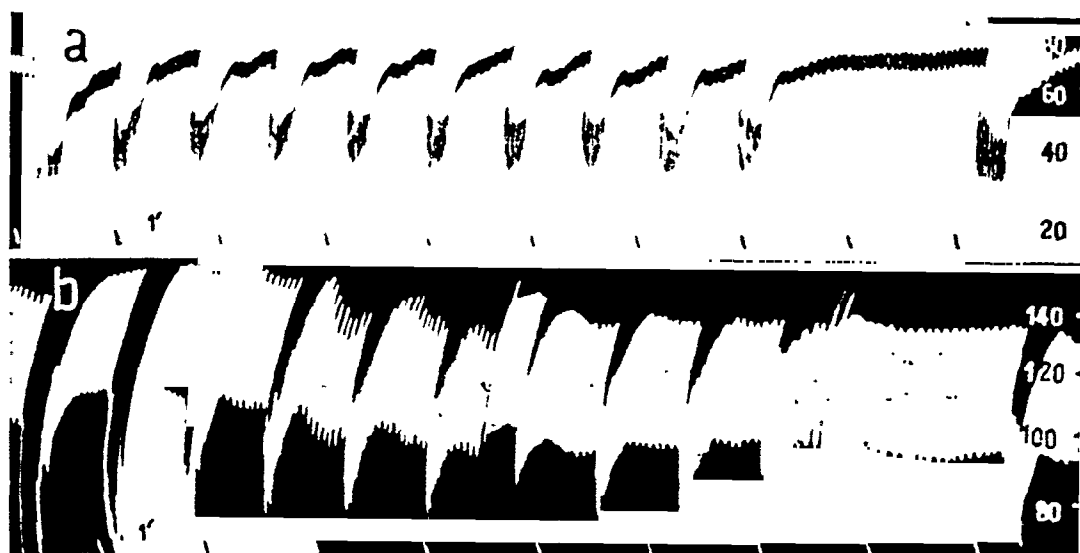


Fig. 1. Cats. Blood pressure records with membrane manometer. Calibration in millimeters Hg. (a) Thyroidectomized 35 days previous to experiment. (b) Injected with thyroxine, 1 mgm. per kgm. daily, for twenty days. Stimulation series of right vagus as described on p. 690.

cats reacted in this way. Figure 1b gives an illustration of such an experiment. In 3 cases the decrease of intensity of the effect of vagus stimulation on the heart was only slightly evident, so that one can say that the hearts reacted "normally" in spite of the hyperthyroid state of the animals. Three cats reacted to vagus stimulation with heart acceleration and slight rise of blood pressure. In 6 of the experiments, there was from the very beginning of the stimulations, and, in spite of the high stimulation intensity, no vagus effect. In 4 of these cases the injections of Prostigmine (200-400  $\mu$ g.) caused the effect of vagus stimulation on the heart to appear. In almost all experiments on hyperthyroid cats a marked rise of pulse pressure was noted, which can be readily explained by the intense peripheral vasodilatation.

*Rats.* The experiments on rats were divided into two groups: (a) Those in which the vagus effect was tested on the heart volume, and (b) those in which

it was tested on the heart frequency. In the first group there were 5 normal and 10 hyperthyroid rats. With the *normal* rats it was noted in all cases that the stimulation series of the right vagus resulted in intense and reproducible heart depressions.

In 7 of the *hyperthyroid* rats the vagus effect was only slightly noticeable at the first stimulation, diminished rapidly and disappeared completely at the end of the stimulation series. After intravenous injection of eserine (20 to 50  $\mu$ g.) there appeared intensive and persisting vagus effects. Three rats of the hyperthyroid series did not react to vagus stimulation and in two of these cases the effect of vagus stimulation on the heart appeared only after injection of eserine. Figure 2 shows one of these cases.

As the opening of the thorax and applying of the cardiometer seemed to us a rather traumatizing procedure, the results were controlled in experiments in which only the heart frequency was recorded. In 10 *normal* rats the intensive vagus effect always obtained decreased only insignificantly at the end of the stimulation series. Of 12 *hyperthyroid* rats 5 did not show any reaction of the heart to vagus stimulation and in 7 the intensity of the effect decreased rapidly,



Fig. 2. Hyperthyroid male rat. Recording of heart volume. Stimulation of right vagus (signals). A: Stimulation with 2.4 V. B: Stimulation with 5 V. Between B and C 50  $\mu$ g. of eserine were given intravenously. C: Vagus stimulation with 2.4 V.

disappearing completely at the end of the stimulations. In 3 cases the vagus effect reappeared after intravenous injection of eserine.

**RESULTS OF SERIES B.** In these experiments 17 thyroidectomized animals (13 guinea pigs and 4 cats) and 47 hyperthyroid animals (22 guinea pigs and 25 cats) were employed. The group of hyperthyroid guinea pigs consisted of 7 animals previously thyroidectomized which received thyroxine 15–20 days after the operation; the rest of the group was formed by animals with intact thyroid glands.

It was regularly observed that the amplitude of the contractions of the isolated hearts of thyroidectomized animals was considerably less than that of hyperthyroid ones (see fig. 3). The frequency was also less.

**Action of adrenaline.** In these experiments the well known fact was confirmed, that the hearts of thyroidectomized animals are only slightly sensitive to adrenaline, while those of hyperthyroid animals become very sensitive to this substance.

In the experiments with *thyroidectomized* cats and guinea pigs, the hearts responded with a moderate increase of frequency and amplitude to doses between 0.05 and 0.1  $\mu$ g. On the other hand the hearts of *hyperthyroid* animals began to react with doses of 0.01 to 0.05  $\mu$ g.

*Depressor ("muscarine-like") action of AC.* The hearts of *thyroidectomized* animals became very sensitive to the depressor effect of AC. Doses of 0.1–1  $\mu\text{g.}$  produced a decrease of amplitude and frequency of the ventricular contractions. With increasing doses of AC (up to 100  $\mu\text{g.}$ ) the negative ino- and chronotrope effect increased progressively.

In hearts of *hyperthyroid* animals, the depressor effect of AC was very slight and sometimes completely absent. Doses of 5–10  $\mu\text{g.}$  of AC usually produced a slight negative chrono- and inotrope effect, which, as the doses of AC were increased to 20  $\mu\text{g.}$ , became less noticeable.

*Stimulating ("nicotine-like") action of AC.* In hearts of *thyroidectomized* animals the stimulating action of AC is almost completely abolished and presents itself in an attenuated form only with high doses of AC (100 to 200  $\mu\text{g.}$ ). In most of the experiments the depressor action of AC was so intense that it did not allow the stimulating effect to appear but this still could be elicited, though with low intensity, when the "muscarine-like" effect of AC was abolished by adding atropine to the perfusing fluid.

The stimulating effect of AC appeared in *hyperthyroid* hearts with doses of between 10 to 30  $\mu\text{g.}$  of AC. This effect accentuated itself progressively as the doses were increased, the depressor effect of AC becoming progressively less intense.

*Liberation of adrenaline.* The dose of 20  $\mu\text{g.}$  of AC, which had, as mentioned before, no stimulating action on the heart of *thyroidectomized* guinea pigs, did not liberate either detectable quantities of adrenaline. The most sensitive preparation of rectal cecums which we have used responded to a concentration of adrenaline of  $10^{-11}$ , so that, if there were any liberation of adrenaline, its concentration must have been below this. With 50  $\mu\text{g.}$  of AC injected into the hypothyroid heart, it was sometimes possible to detect amounts of adrenaline equivalent to concentrations of  $10^{-11}$  to  $4 \times 10^{-11}$ . A 100  $\mu\text{g.}$  dose of AC given to the heart always led to liberation of adrenaline the concentrations of which were of the order of  $10^{-10}$  to  $4 \times 10^{-10}$  though in some cases the injection of 100  $\mu\text{g.}$  of AC had no stimulating effect on the heart.

Figure 3 shows the effect of a dose of 50  $\mu\text{g.}$  of AC injected into the heart of a thyroidectomized guinea pig (in (1)). Ten cubic centimeters of perfusate are collected between the marks, and employed to irrigate the rectal cecum in (a). It can be seen that 50  $\mu\text{g.}$  of AC did not liberate detectable quantities of adrenaline (less than 0.0001  $\mu\text{g.}$  per cc.).

Twenty micrograms of AC injected into *hyperthyroid* hearts liberated quantities of adrenaline which were perfectly detectable on the rectal cecum of the fowl and which varied in concentration between 0.00015 to 0.0002  $\mu\text{g./cc.}$  Fifty micrograms of AC liberated greater amounts of adrenaline which were equivalent of concentrations of  $2 \times 10^{-10}$  to  $6 \times 10^{-10}$ .

In figure 3 the action of the perfusate of a hyperthyroid heart, collected between the signals in (I) is to be seen in (b). The effect is equivalent to a concentration of adrenaline of  $4 \times 10^{-10}$ .

The injection of 50  $\mu\text{g.}$  of AC into a hyperthyroid heart almost always elicited

a maximum of adrenaline liberation. Higher doses of AC very seldom liberated higher concentrations of adrenaline.

**DISCUSSION.** The disturbances of the heart reaction to vagus stimulation, which were observed in hyperthyroid animals, could originate from the following causes:

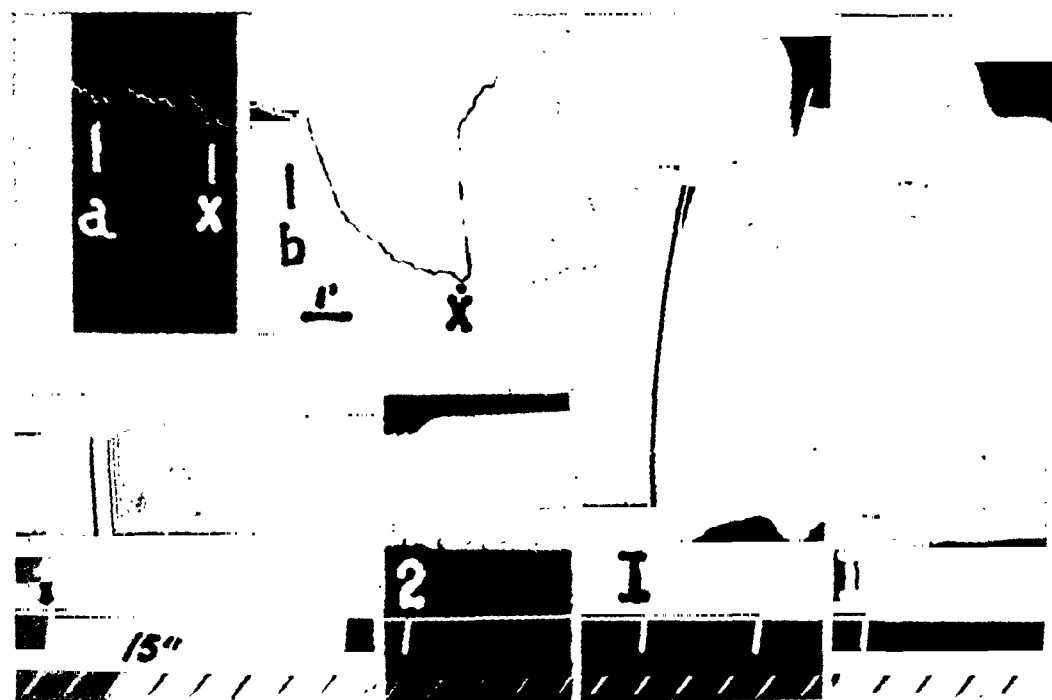


Fig. 3. Response of two isolated guinea-pig hearts to acetylcholine and epinephrine and detection in the heart perfusates of and epinephrine-like substance liberated by the action of acetylcholine (as described on p. 691).

*The upper record on the left.* Isolated rectal cecum of a chicken irrigated, before (a), before (b) and after (x), with atropinized Tyrode solution containing 5 µg. of acetylcholine per cubic centimeter.

*The lower record on the left.* Ventricular contractions of a thyroidectomized guinea-pig's heart. At (1) 50 µg. of acetylcholine were injected. Between the signals 10 cc. of perfusate were collected and given, after atropinization, to the fowl's intestine, in (a). At (2): action of 0.1 µg. of epinephrine.

*The record on the right.* Ventricular activity of a hyperthyroid guinea-pig's heart. At (I) 50 µg. of acetylcholine were injected. Between the signals 10 cc. of perfusate were collected, atropinized, and given to the rectal cecum, in (b). At (II) injection of epinephrine, (0.05 µg.).

The relaxation of the rectal cecum was equivalent to one produced by Tyrode solution containing 0.0004 µg. epinephrine per cubic centimeter.

Both guinea pigs had been thyroidectomized 18 days previous to the experiment and one of the animals (right record) had received 3.7 mgm. of thyroxine in daily doses of 1 µg. per gram until the metabolic rate had risen from 36 to 71 Calories sq. metre/hr.

(1) The transmission in the cardiac fibres of the vagus could be impaired, (2) there could exist a blockage of the synaptic transmission, (3) the concentration of AC which is liberated at the vagus endings could be insufficient, (4) there could exist a desensitization of the heart fibre to AC. This last assumption is supported by our experiments on the isolated heart of hyperthyroid animals, which

clearly show a decreased response of the heart muscle to the depressor effect of AC. Therefore one may compare the condition of the hyperthyroid heart with a kind of curarisation, more so as it is shown in the experiments of series A that, in hyperthyroid cats, rabbits and rats, eserine and prostigmine may restore a diminished or even completely absent heart effect of vagus stimulation. The antagonism between curare and eserine is a well established fact (14, 17). In the normal animal, Mautner and Luisada (12) produced a heart vagus block by injecting curare, this block being easily caused to disappear by eserine.

In an earlier paper it was shown that in the hearts of normal mammals there exist at least two types of cholinergic effectors: the heart fibre on which AC exerts a depressor action and structures which are most likely of nervous origin (sympathetic ganglia or chromaffine tissue), the stimulation of which by AC leads to liberation of adrenaline. The appearance of this agent explains the stimulating effect of AC (10).

From the above described experiments it can be seen that the sensitivity of the cholinergic heart effectors is altered under the influence of a lack or an excess of thyroid hormone. In thyroidectomized animals the myocardium becomes highly sensitive to the depressor action of AC and slightly sensitive to the stimulating effect, the latter fact being due to a desensitization of the ganglionic structures of the heart to AC. This statement is based on the above described experiments which show that, in hypothyroid hearts, the quantity of adrenaline liberated by a given dose of AC is considerably less than that which appears with the same dose of AC in the perfusates of hyperthyroid hearts. High doses of AC given to a hypothyroid heart will liberate detectable quantities of adrenaline. But often the hearts, even after atropinization, do not respond with stimulation to these doses of AC, due to their low sensitivity to adrenaline. The fact of an impaired response of the hypothyroid heart to adrenaline has been already described (6, 18, 19, 20) and has been fully confirmed in our experiments.

The changes of sensitivity of the heart structures which appear in hyperthyroidism are a perfect antithesis of those observed in athyroid animals. The sensitivity of the hyperthyroid myocardium to the muscarine-like action of AC is greatly decreased while the "ganglionic" structures are abnormally easily stimulated, leading thus to liberation of unusually high quantities of adrenaline. Besides, the myocardium becomes highly sensitive to adrenaline, a phenomenon that has been already described (2, 8, 11, 15, 18, 21) and has been confirmed by us.

So far, we have no direct proof that AC which is normally liberated at the vagus endings may exert any influence on intracardiac ganglionic structures. Yet we cannot reject this possibility, the more so because it is known that, in other structures, as for instance in the sympathetic ganglion (4, 5), the stimulation of the preganglionic fibres or injection of AC into the ganglion, lead to liberation of adrenaline in the ganglion itself. In small concentrations this substance seems to facilitate ganglionic transmission; in high concentrations, to abolish it. It is not impossible that such a mechanism may exist in the heart and that the intracardiac nervous apparatus is constantly under the influence of AC, modulating thus the sensitivity of all heart effectors.



Undoubtedly, the thyroid hormone interferes profoundly in the interplay of AC and adrenaline. Thus the "paradoxical vagus effect" described on p. 692 and which is sometimes observed in hyperthyroid animals, may be due to the liberation of AC at the vagus endings, which stimulates nervous structures leading therefore to liberation of adrenaline.

The experimental results described above contribute to elucidate the mechanism of the heart symptoms observed in thyroid disorders. We may assume that the changes of sensitivity of adrenergic and cholinergic heart effectors to the respective neurotransmitters, caused by lack or excess of thyroid hormone, contribute largely to the appearance of bradycardia or tachycardia respectively.

The fact that the effector systems of the heart are influenced in such a pronounced way by the thyroid hormone makes it possible to believe that similar changes may also occur in other neuroeffectors leading thus to the production of other symptoms of thyroid disorders.

#### SUMMARY

(1) In hyperthyroid rabbits, cats and rats the effect of vagus stimulation on the heart is less marked than in normal animals. Often there is no vagus effect at all and sometimes the stimulation of the vagus leads to cardio acceleration.

(2) The impaired vagus effect is returned to normal by eserine or prostigmine.

(3) In thyroidectomized cats the effect of vagus stimulation on the heart is more marked and maintained than in normal animals.

(4) In isolated hearts of hyperthyroid cats and guinea pigs, acetylcholine has only a slight depressor action. This effect is always followed and overshadowed by a pronounced stimulation, similar to that produced by epinephrine.

(5) In perfusates flowing from hyperthyroid hearts there appear, as a consequence of injections of relatively low doses of acetylcholine, unusually high amounts of an epinephrine-like substance.

(6) On isolated hearts of thyroidectomized cats and guinea pigs, AC exerts an intense depressor effect. The stimulating action of this drug is very weak and often completely absent.

(7) In perfusates of isolated hearts of hypothyroid guinea pigs there appears an epinephrine-like substance only when high doses of AC are injected.

(8) A high sensitivity of the hyperthyroid heart to adrenaline and a low sensitivity to this agent of the myocardium of hypothyroid animals is always observed.

(9) The changes of the sensitivity of the heart effector systems to the neurotransmitter substances contribute to the elucidation of the cardiac symptoms observed in thyroid disorders.

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# FURTHER STUDIES ON ANTIGONADOTROPHIN FORMATION FOLLOWING GONADOTROPHIC HORMONE ADMINISTRATION<sup>1,2</sup>

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Sheep anterior pituitary extracts alone and in combination with human chorionic gonadotrophin are reported to have a stimulating effect on the human ovary (1, 2). However, allergic manifestations have been noted during their clinical use and antihormone formation may occur following protracted treatment with the combination of gonadotrophins, Synapoidin (3). Further information concerning the antihormonic action of clinically available preparations is sought by studies in rabbits since the development of antihormones in rabbits may indicate the possibility of such occurrences in man. Antihormones and an antibody titre against the combination of sheep anterior pituitary extract and human chorionic gonadotrophin have been found to develop readily in the rabbit (4, 5), thus simulating the antigonadotrophic formation noted with crude sheep pituitary material (6). In this investigation we have extended our studies on the combination of gonadotrophins to include 1, the route of administration and antihormone formation; 2, the rate of antihormone disappearance; 3, the effect of hormone reinjection; 4, the antigenicity of the gonadotrophin of low potency due to aging, and 5, the ability of anti-Synapoidin serum to antagonise gonadotrophins from other sources.

**MATERIALS AND METHODS.** For these experiments 14 male rabbits weighing 2.8 to 3.6 kgm. were used. Each animal was injected once daily with either 1.5 r.u. (0.1 cc.) or 4.5 r.u. (0.3 cc.) of the combination of gonadotrophins (Synapoidin)<sup>3</sup> either subcutaneously or intravenously, the extract containing 0.7 mgm. nitrogen per cubic centimeter. The hormone was administered on the first four days of each week for 3 successive weeks. Then, 6 to 8 days after the last injection or days 24 to 26 of the experiment, a 10 cc. blood sample was drawn from the heart. Following a 10 to 20 day interval after obtaining the blood sample, a second series of injections was administered in the manner indicated but extending over only a 2-week period. The animals were bled 7 to 10 days after the last injection. Blood samples were again obtained at a time when the antigonadotrophins had disappeared in 7 of 8 rabbits and

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<sup>3</sup> Grateful acknowledgment is made to Dr. D. A. McGinty of Parke, Davis and Company for the Synapoidin (a combination of sheep anterior pituitary extract and human chorionic gonadotrophin containing 15 r.u. per cc.); to Dr. B. J. Brent of Roche-Organon, Inc. for the human chorionic gonadotrophin, Pregnyl; and to Dr. D. H. Wonder of Cutter Laboratories for the pregnant mare serum, Gonadin.

6 of these animals were then reinjected in the manner just described. Therefore, only 3 blood samples were drawn from each rabbit over the 3-month period of the initial injection series.

The presence of antigonadotrophins in the serum was estimated by determining the degree to which the serum inhibited the ovarian weight increase anticipated with the gonadotrophin alone. For these tests, 22-day-old female rats or mice were used. All of the animals received a total of 3 r.u. of Synapoidin and 2 of the 4 littermates received in addition, at another site, a total of 0.9 cc. of the serum to be tested. Injections were made subcutaneously once daily for 3 days and the animals were killed 24 hours after the last injection. The weights of the ovaries and uteri were obtained, the latter after removal of intrauterine fluid. Some tests were performed with 22-day-old male rats and mice in which the same total dosages of hormone and serum were used as previously mentioned but the injection period was extended to 4 days. Pregnant mare serum, human chorionic gonadotrophin, human pituitary and the pituitary from adult male rats were also tested against anti-Synapoidin serum. The water soluble portion of acetone dried human pituitaries was used. The rat pituitaries were prepared and administered as previously described (7), a method simulating that of Reece and Weatherby (8).

**RESULTS AND DISCUSSION.** The influence of the route of injection on anti-hormone formation was investigated in 8 rabbits. All were tested prior to hormone administration and the serum, in each case, failed to cause any diminution of the gonad stimulating activity of the hormone. The rabbits were divided into two groups, one group was injected subcutaneously and the other group injected intravenously. A daily dose of 4.5 r.u. (0.3 cc.) was administered to all animals. After 3 weeks, the sera from 3 of 4 rabbits injected subcutaneously had a marked antigonadotrophic activity whereas serum from each of the 4 rabbits injected intravenously failed to reveal the presence of antigonadotrophins (table 1). Injections were continued in each group for an additional 2 weeks, after which time the animals injected subcutaneously exhibited a marked antihormone titre in all cases but the sera from only 2 of the 4 rabbits injected intravenously exhibited antihormone titres and then of only modest strength. It is evident that inhibitory substances against the administered gonadotrophin are formed more readily when the subcutaneous injection route is used if the gonadotrophin is a combination of sheep anterior pituitary extract and human chorionic gonadotrophin (table 1).

Antihormone disappearance time was estimated by drawing serum samples between 21 and 40 days after the last hormone injection and it was found that the antigonadotrophins had disappeared by this time in 5 of the 6 rabbits previously exhibiting these hormone antagonists. The exceptional case was a rabbit that had a high titre 33 days after the last injection (table 1).

The influence of a reinjection series was studied to determine whether antihormones would form more readily in those animals previously producing antihormones and whether antihormones would now develop in animals not responding to the initial injection series (2 rabbits injected intravenously).

The serum from each rabbit was tested prior to this restudy to assure the absence of antigonadotrophins. A period of 40 to 62 days intervened between the two injection series and a period of 17 to 30 days was permitted to elapse between the time when antihormones were no longer detectable and the first injection of the reinjection series was given. Rabbits 3 and 6 (tables 1 and 2) were again injected subcutaneously with the 4.5 r.u. daily dosage and a reformation

TABLE 1  
*Mode of administration and antigonadotrophin formation in the rabbit*

ANIMAL NO.	SERUM TEST—DAYS AFTER FIRST INJECTION (SERUM INHIBITORY ACTION)			
Gonadotrophin injected subcutaneously				
3	24 (—)	55 (+)	84 (—)	
6	24 (+)	54 (+)	68 (—)	96 (—)
9	26 (+)	49 (+)	75 (+)	
10	24 (+)	60 (+)	93 (—)	
Gonadotrophin injected intravenously				
2	24 (—)	55 (—)	84 (—)	
7	24 (—)	54 (+)	68 (—)	96 (—)
8	26 (—)	49 (+)	75 (—)	
11	24 (—)	60 (—)	93 (—)	

(+) = inhibitory, (—) = negative.

All rabbits injected with 4.5 r.u. daily 4 days of each week.

First bleeding after a 3-week injection period. Second bleeding after a 2-week injection period.

of antihormones resulted in 3 weeks. The response to the reinjection series appeared to be elicited more readily than when the hormones were first administered. This is indicated in rabbit 3 in which a definite antihormone titre was developed in 3 weeks of the reinjection period whereas the animal had failed to respond in this period of time during the initial injection series. Furthermore the rate of antihormone disappearance was less rapid following the reinjection series as compared with the initial injection series as indicated

by rabbit 6. The serum from this animal contained antigonadotrophins 45 days after the last injection of the reinjection series whereas antihormones were not present in the serum obtained only 21 days after the last injection of the initial series (tables 1 and 2).

Rabbits 2, 7 and 8 had previously been injected intravenously and in two of these a partially inhibitory serum had developed. Rabbit 2 which had failed to develop antihormones previously was now injected subcutaneously and a strongly inhibitory serum developed after 3 weeks. Rabbit 7 responded in similar fashion and in this animal the antihormones persisted for 45 days without decline (table 2). Finally it was of interest to study the response of the rabbit when the gonadotrophins were given intravenously in both injection series. One rabbit (no. 8) had developed a low antihormone titre during the

TABLE 2

*Reinitiation of antigonadotrophins in the rabbit with gonadotrophins*

ANIMAL NO.	SERUM TEST—DAYS AFTER FIRST INJECTION (SERUM INHIBITORY ACTION)			
2	29	61		
	(+)	(+)		
3	29	43	57	
	(+)	(+)	(-)	
6	31	49	62	86
	(+)	(+)	(+)	(+)
7	31	59	72	96
	(+)	(+)	(+)	(+)
8*	24	48		
	(+)	(+)		

\* Injected intravenously, all others subcutaneously. Last hormone injection between days 40-45.

initial injection series. A month later when the antihormones had disappeared, this rabbit was reinjected intravenously for 3 weeks with the 4.5 r.u. daily dose following which a strongly antigonadotrophic serum was obtained (table 2). Therefore, antigonadotrophins against the gonadotrophin used appear to form more readily and persist for a greater length of time in response to a reinjection series.

The formation of antigonadotrophins in response to a smaller daily dosage than that used previously was studied in 2 rabbits. These animals were injected subcutaneously with 1.5 r.u. (0.1 cc.) daily in the manner described and after 3 weeks of hormone administration (day 24 of the experiment) antigonadotrophins could not be demonstrated. However, following the additional 2 week injection period a strongly inhibitory antigonadotrophic serum was obtained from both animals. On follow-up, it was found that antigonadotrophins had

disappeared 26 days after the last injection and therefore a reinjection series was begun 13 days after the serum was found to be negative. The same daily dosage was again administered and this time antihormones were present in the serum after 3 weeks of injection as contrasted with the initial injection series in which antihormones failed to develop in 3 weeks. These results are similar to those obtained with the 4.5 r.u. daily dosage.

Chase (4) has demonstrated that the antibody titre can be correlated with the antihormone titre in serum from rabbits injected with the same gonadotrophic preparation used in this study. The precipitin tests measure sensitivity and not the amounts of reaction concerned. Therefore, the Libby photoreflexometer which has been used by Boyden (9) in many studies in serology in the measurement of amount of reaction, was used to test the serum from a rabbit which had a marked inhibitory effect physiologically. This serum failed to provide evidence for the presence of antibodies by the method used.

Extracts containing gonadotrophic hormones are known to lose potency when left in solution even in the cold and these extracts will rapidly deteriorate when kept at room temperature. How this aging process influences the ability of the same solution to elicit antigonadotrophin formation has been touched upon by Twombly (10) who found human chorionic gonadotrophin would develop antihormones in the rabbit even though the material was 3 years old and contained no demonstrable activity. Zondek and Sulman (11) suggest that the material was not completely inactive in that pregnancy urine inactivated by heat will not cause antigonadotrophin formation. The antihormone forming ability of a gonadotrophic extract in solution was tested after its potency had been reduced on standing at room temperature for 17 months, our estimations of gonadotrophic content being  $\frac{1}{5}$  to  $\frac{1}{8}$  of that initially present. Two rabbits were injected subcutaneously with 0.3 cc. daily and 2 animals received 0.1 cc. daily on 4 days of each week. On day 24, after 3 weeks of hormone administration, a strongly inhibitory serum was obtained in all cases. Continued hormone administration for 2 weeks led to the continued presence of antihormones which, however, were no longer detectable 34 days after the last injection. These results simulate those obtained with the potent preparations and despite the loss in gonadotrophic activity no evidence of a decrease in ability to form antigonadotrophins is indicated.

Unlike pregnant mare serum and chorionic gonadotrophin against which relatively specific antigonadotrophins are formed, a crude sheep pituitary extract excites the formation of a nonspecific inhibitory serum (12-14). Since a combination of sheep anterior pituitary extract and human chorionic gonadotrophin comprised our injected hormone it seemed desirable to test for a nonspecific action of antigonadotrophic serum formed against this material. Tables 3 and 4 illustrate the nonspecific nature of the antigonadotrophic rabbit serum as tested in male and female rats and mice. In the female, the serum will not only inhibit the gonadotrophic action of the gonadotrophins injected but exerts the same effect against pregnant mare serum, human chorionic gonadotrophin, male rat pituitary and human pituitary. Similar results were reported with

TABLE 3  
*Non-specific nature of antigonadotrophic rabbit serum*

HORMONE (TOTAL DOSE)	AVERAGE OVARIAN WEIGHT		TEST
	Hormone	Hormone and sera	
Tests on rats			
Synapoidin (3 r.u.) .....	50	15	+
P.M.S. (10 I.U.).....	28	14	+
Rat AP (1 male).....	63	14	+
Human AP (2 mgm.).....	66	16	+
Tests on mice			
Synapoidin (3 r.u.).....	8.4	2.3	+
P.M.S. (10 I.U.).....	7.4	1.8	+
P.U.E. (40 I.U.).....	6.3	1.9	+

Ovarian weight of control rats 12.8 mgm., of mice 3.1.

P.M.S. = pregnant mare serum.

P.U.E. = human chorionic gonadotrophin.

Synapoidin = sheep anterior pituitary and human chorionic gonadotrophin.

TABLE 4  
*Tests with antigonadotrophic rabbit serum*

HORMONE (TOTAL DOSE)	AVERAGE ORGAN WEIGHT (MGM.)		TEST
	Testis	Seminal vesicles	
Tests on rats*			
Synapoidin (3 r.u.).....	664	52 ± 2.0†	+
Synapoidin and serum.....	564	21 ± 1.3	
Rat AP (2♂).....	645	26 ± 1.0	—
Rat AP and serum.....	469	26 ± 2.1	
Controls.....	350	13 ± 0.7	
Tests on mice*			
Synapoidin (3 r.u.).....	106	37 ± 2.2	+
Synapoidin and serum.....	113	11 ± 1.8	
P.M.S. (10 I.U.).....	82	21 ± 1.0	+
P.M.S. and serum.....	27	6 ± 0.8	
P.U.E. (40 I.U.).....	110	22 ± 1.1	+
P.U.E. and serum.....	60	8 ± 0.7	
Controls.....	55	10 ± 0.5	

\* Four animals in each group.

$$\dagger \epsilon = \sqrt{\frac{d^2}{n(n-1)}}$$

the antiserum against sheep pituitary extract (12). Virtually all of the inhibitory tests with antigonadotrophic sera are made in the female and for that



reason the tests were extended to include the male. These antihormone tests in male animals were performed in essentially the same manner as in females. Eight animals were injected with the gonadotrophin and of these 4 received in addition 0.9 cc. of serum. The inhibitory action of the serum was indicated by a failure of seminal vesicle weight to increase to the same degree as with the gonadotrophin alone. A significant inhibitory action of the serum was indicated in tests against the combination of gonadotrophins (Synapoidin), pregnant mare serum, and human chorionic gonadotrophin but the activity of the male rat pituitary was not influenced.

All of the antihormone tests indicating its nonspecific gonadotrophic nature were made with the serum from one rabbit that had received two of the 5 week injection series. Since source specificity may have been lost due to the extended injection period some of these studies were re-examined with the serum from a rabbit given only one of the 5 week injection periods. For added interest the antiserum was obtained from a rabbit that had received a 0.3 cc. daily dose of the extract with reduced gonadotrophic potency due to aging. This anti-gonadotrophic serum proved capable of inhibiting the ovarian stimulating action of Synapoidin, pregnant mare serum and human pituitary. In the male, the activity of pregnant mare serum and Synapoidin was counteracted by the serum but male rat pituitary activity was not altered. Although many other gonadotrophins could have been tested, the range of sources used provides a definite indication of the nonspecific inhibitory action of this rabbit serum.

Treatment of the immature rat and of the dog with antigonadotrophic serum causes atrophy of the gonads and castration-like changes in the pituitary gland (6, 15, 16). In order that the effect of antigonadotrophic serum could be observed in mice, we injected 5 male mice, 24 days of age, subcutaneously with 0.3 cc. daily for 12 days and killed the animals 24 hours after the last injection and compared them with 5 littermate controls. Body weight increase averaged 8.4 grams in each group. However, the testes from the serum treated mice averaged only 24 mgm. and were devoid of spermatozoa whereas the testes from the controls averaged 99 mgm. and all exhibited spermatozoa. The seminal vesicles too reflected the effect of the serum in that these organs averaged only 7 mgm. in the serum treated group as compared with 26 mgm. in the control group. Furthermore, the adrenal X-zone which normally disappears with the maturation of the male reproductive system and was absent in the controls was found to be present in the serum treated mice, a further indication that androgen was not being secreted.

#### SUMMARY

Antigonadotrophin formation against a combination of sheep anterior pituitary extract and human chorionic gonadotrophin (Synapoidin) could be induced more rapidly in the rabbit by the subcutaneous route than by the intravenous route of administration. These inhibitory substances generally disappeared within 20 to 40 days. Animals that were given a reinjection series demonstrated that

antigonadotrophins form more readily and persist for a greater length of time following hormone administration in a reinjection series.

Despite the reduction in gonadotrophic potency induced by standing at room temperature, no evidence of a decrease in ability to elicit antihormone formation was indicated.

The non-specific inhibitory nature of the antigonadotrophins was shown by the ability of the serum to nullify the gonadotrophic action of pregnant mare serum and human chorionic gonadotrophin in the male and female rat. In addition, human pituitary and male rat pituitary extracts were antagonised in the female rat but the male rat pituitary solution was not inhibited in the male test animal. A non-specific inhibitory serum was obtained from the rabbits injected with the gonadotrophic extract of reduced potency due to aging.

A pronounced inhibitory effect on the reproductive system of male mice was observed following the administration of the antigonadotrophic serum.

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# STUDIES ON PERMEABILITY OF THE LEUCOCYTE

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Large amounts of carefully controlled experimentation have been done on permeability of mammalian red blood cells (1-8), various marine life (9-11), and more recently tissue systems (12-13). The leucocyte, however, has with few exceptions (14-15) been singularly neglected. Various reasons are advanced to account for the paucity of studies on white cell permeability and fragility: 1. The relatively short survival of white cells under "stored" conditions. 2. The existence of three morphological types of white cell. 3. The inability of white cells to withstand experimental "handling". 4. The absence of hemoglobin or other cellular pigment whose escape would serve as a handy index of cell lysis.

Shapiro and Parpart (14) studied the volume changes of leucocytes in hypotonic media and recorded swelling to as high as 1000  $\mu^3$  following exposure to 0.4 per cent Ringer-Locke solution. They noted a reversibility of the process following alteration from hypotonic to hypertonic solution. On the basis of shrinkage of cell size back to normal range, they postulated that white cells were not visibly damaged by exposure to hypotonic media. Their observations covered a relatively brief time interval. Their criteria of absence of cell damage were the ability to undergo a reversal of volumetric change, and constancy of the total count.

If any correlation exists between red and white cells, it is not difficult to conceive an altered membrane permeability prior to cell lysis. Jacobs, Glassman and Parpart in 1933 (3), Ponder in 1933 (5) and Davson in 1937 (16) demonstrated with erythrocytes that enough stretching of the membrane occurs in hypotonic solution to permit potassium leakage prior to hemolysis.

In studies on permeability of any cell type or tissue system, it is desirable to maintain constant environmental conditions of temperature, pH, and oxygen tension. Recent observations with banked blood (18) seem to indicate that oxygen tension is of relatively minor effect in leucocyte survival. The bulk of evidence, however, points to its being of definite import in permeability studies.

**OBJECT.** It was the object of this series of experiments to observe the permeability rate of normal leucocytes to water as reflected by their ability to withstand lysis in varying ranges of anisotonic media, over stated time intervals. It was hoped, thereby, to: 1, establish a base line of normal leucocyte fragility, for comparative use in experiments (to be reported separately) on the permeability of leukemic leucocytes, 2, determine whether different cell types, i.e. lymphocytes as opposed to granulocytes, would show predilection for different osmotic tensions.

<sup>1</sup> Donner Foundation, Research Fellow.

**METHOD.** All experimentation was performed on leucocytes which were maintained as living cells in tissue culture technique.

The permeability of the cells to water was recorded in terms of presence or absence of lysis following exposure to graded degrees of anisotonia over stated time intervals. The presence or absence of lysis was determined by recording serial absolute counts, through combined differential smears and total white counts.

*Description of techniques.* The culture technique of Osgood and Brownlee (17) was used with minor variations. Cultures were kept in 30 cc. pyrex vaccine vials capped with rubber stoppers and all transfers of cells, media or gas were made with sterile syringes and needles. All cultures were incubated at 37.5°C. The gas phase was changed q. 12.h. The pH was checked at the beginning and end of each experiment.

The media consisted of a mixture of Parker salt solution (21), and sterile cord serum in the proportions of  $\frac{2}{3}$  to  $\frac{1}{3}$ . The cord serum was collected by the technique of Israels (20).<sup>2</sup> After collection, freezing point determinations were run on the pooled serum to determine its total milliosmolar strength. It was then sterilized by passage through ultrafine glass filters and collected in sterile vials.

**PROCEDURE.** The white cells for each experiment were collected and washed as follows. Fifty milliliters of blood was withdrawn from normal female donors and divided between 2 sterile 60 cc. bottles containing 25 cc. each of isotonic salt solution, and 4 mgm. of heparin. These were centrifugated at 2000 RPM for 20 minutes and 25 cc. of supernatant fluid removed and discarded. The remaining contents were then resuspended and transferred to two 25 cc. Cushman tubes of proper hematocrit size, for centrifugation at 2500 RPM. At the end of 30 minutes the buffy coat was layered in the central capillary portion of the tubes. It was extracted with a sterile 6-inch needle and placed in small sterile test tubes containing 5 ml. of isotonic saline. These were briefly centrifuged (3-5 min.) and the washed white cells were then removed for use.

A series of vaccine vials numbered 1 through 7 was set up for each experiment. Each vial contained 3 cc. of media, consisting of  $\frac{2}{3}$  Parker salt and  $\frac{1}{3}$  isotonic cord serum. The Parker salt solution in each vial was altered by dilution or concentration in such a way that the total milliosmolar content of the media was of the following order: Vial 1, 0.237 m; vial 2, 0.270 m; vial 3, 0.300 m; vial 4, 0.320 m; vial 5, 0.337 m; vial 6, 0.360 m; vial 7, 0.380 m.

The various electrolytes were kept in constant proportion to one another. Only the total milliosmolar strength was varied. A series of tonicities was thus established, consisting of three vials of hypotonic media, one of isotonic and three of hypertonic media. Quantitatively similar amounts of washed white cells from a single donor were then added to each of the seven vials, until the white count in each vial was 8000 c.m.m. The vials were then incubated and observed for 30 hours. At stated time intervals:  $\frac{1}{4}$ , 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 24 and

<sup>2</sup> The author is indebted to Miss Kellerer of the Boston Lying In Hospital, Blood Bank, for aid in collection and centrifugation of the placental blood.

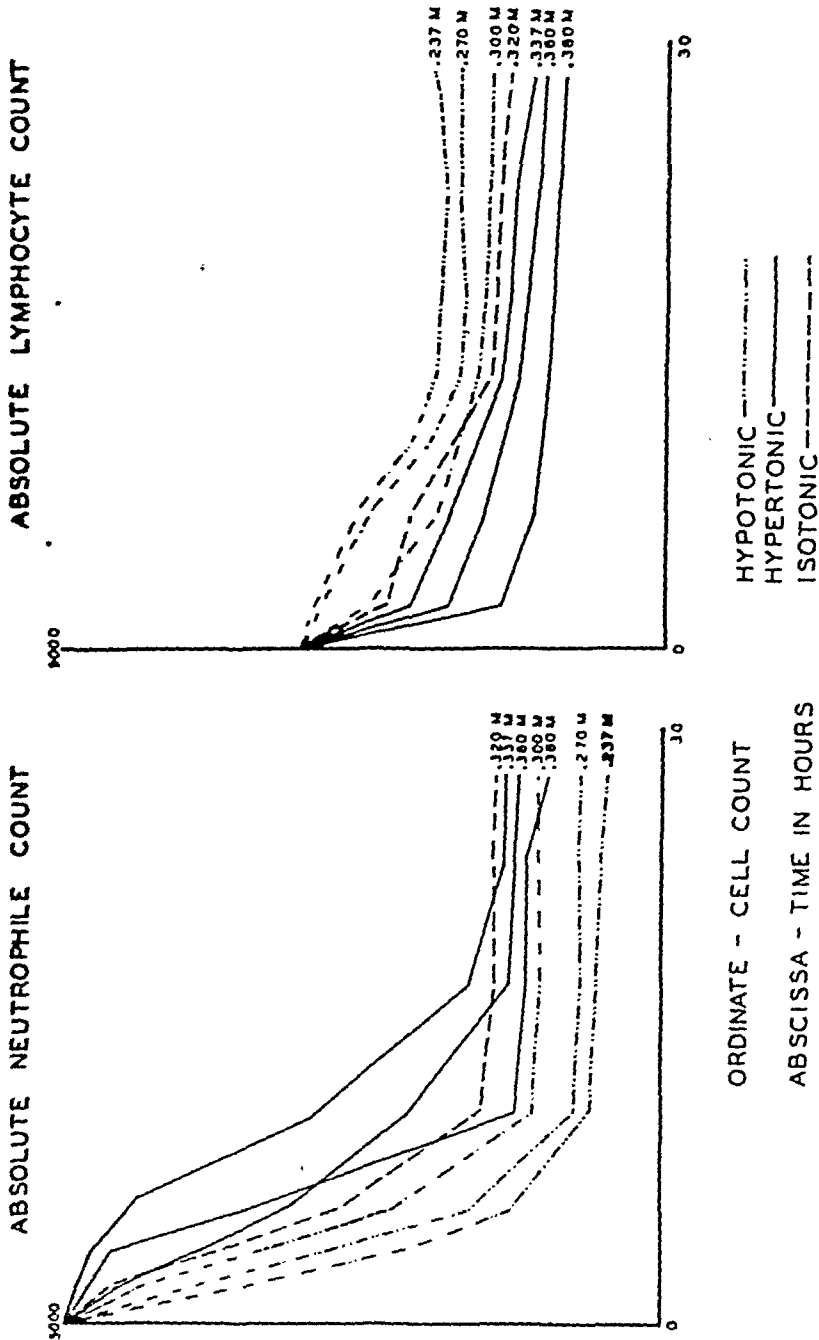


Fig. 1

30 hours, subsamples were removed for differential smears and total white counts. Prior to removal of samples, the vials were placed on a rotar and spun for 15 minutes to insure uniform cell suspension.

**FINDINGS.** Studies were carried out on fifteen normal donors. In each case the leucocytes were separated from the whole blood, washed, resuspended as outlined, and observed for 30 hours.

The largest amount of cell lysis for all types of cells occurred during the first 1 to 3 hours. Following this early drop in total white count there was a grad-

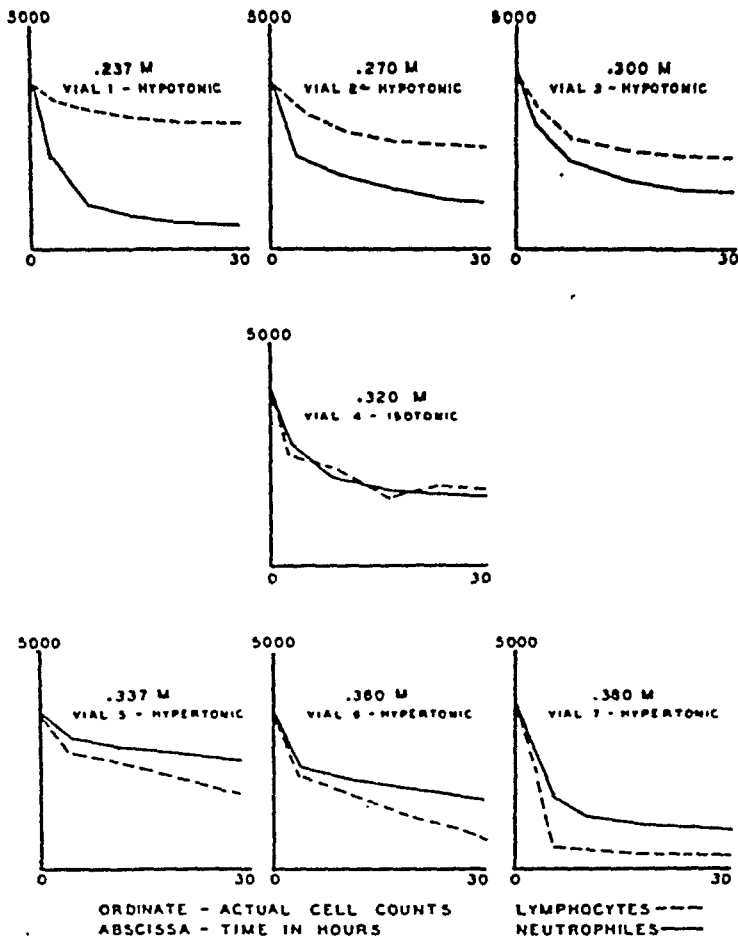


Fig. 2

ual fall over the remaining 30 hours. Beyond 30 hours, a few cells (chiefly lymphocytes) were invariably present, but they were too fragmented to warrant further differential counting. Cell distortion and large numbers of so-called basket cells tended to appear on the stained smears after 5 hours in culture. However, it was found that such distortion and smudging could be overcome almost completely by rapid drying of the smears. If the pulled smears were fixed immediately in absolute methyl alcohol, cellular outlines and staining properties remained sufficiently normal to permit a high degree of accuracy in differential counting.

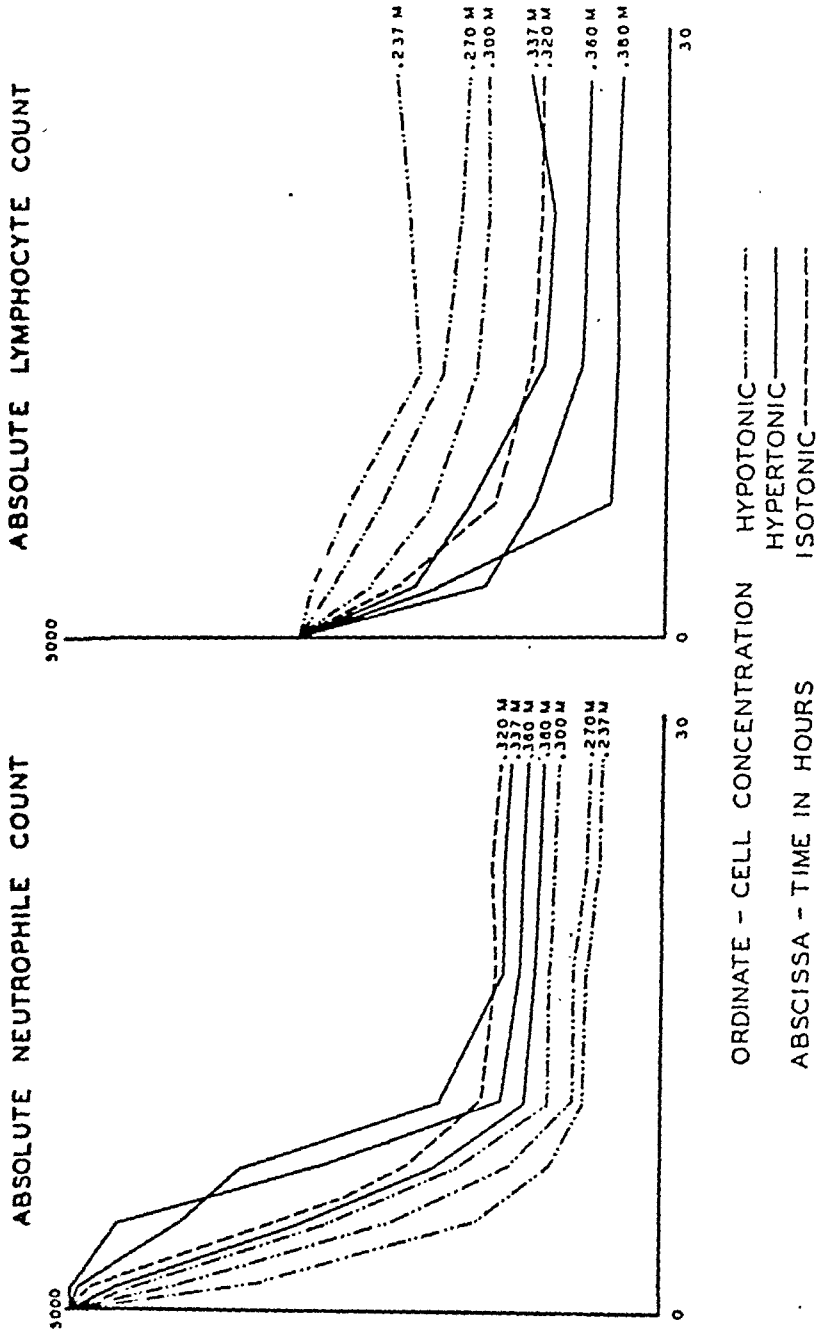


Fig. 3

It was found that leucocytes are able to withstand a molarity ranging between 237 milliosmoles and 380 milliosmoles. It was found that lysis of lymphocytes, in terms of reduction in absolute numbers, occurred least often in hypotonia. In general, the greater the hypotonia (i.e., less the molarity), the greater the number of intact lymphocytes remaining. It was found that lysis of polymorphonuclear neutrophiles, in terms of reduction in absolute numbers, occurred least often in isotonia and slight hypertonia (320–337 milliosmoles).

It was found that eosinophiles were apparently unaffected by anisotonia of the strengths employed in these experiments. Their absolute numbers were generally the same at the conclusion of the experiments as at the beginning. The preferential affinity of lymphocytes for hypotonia and neutrophiles for hypertonia can be well seen if one plots the absolute cell counts against the time intervals at which the readings were made. Figure 1 (expt. G.) is cited as a typical case.

In another experiment (E), the donor's blood happened to contain equal numbers of lymphocytes and granulocytes at the start of the experiment. If, for that experiment, one plots a separate graph for each molarity, the differential effect on lymphocytes and granulocytes is again illustrated.

In 13 of the 15 experiments, lymphocytes showed a definite predilection for hypotonia and granulocytes for hypertonia. Although this affinity was, at times, not marked, it was none the less a real and constant finding. In the other two experiments, there was uniformly poor polymorphonuclear survival in all seven culture vials, regardless of tonicity. No explanation is offered for failure of these two experiments to correspond to the general pattern. If a summation of the results from all 15 experiments is made, the resulting graphs are seen to conform to the general pattern indicated in experiment G, figure 1.

No attempt was made to study quantitatively the resistance of monocytes or basophiles.

#### SUMMARY

Washed white cells were suspended under sterile conditions in tissue media of varying tonicity. It was found that:

1. Leucocytes can exist in tonicities varying between 220 and 380 milliosmoles (about 0.65 per cent to 1.25 per cent NaCl).
2. Polymorphonuclear neutrophiles retain their morphological integrity longest in isotonia and slight hypertonia (320 to 337 milliosmoles).
3. Lymphocytes retain their morphological integrity longest in hypotonia.
4. Eosinophiles are "hardy" cells and are apparently unaffected by anisotonia between 237 and 380 milliosmoles.

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# THE DIFFERENCE IN ACTION OF PARASYMPATHETIC DRUGS IN APPLICATION TO SEROSA OR MUCOSA OF THE ISOLATED INTESTINE

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Many drugs are less efficacious when given orally than parenterally, which fact is usually explained by either digestive destruction or lack of absorption due to impermeability of the intestinal wall. In experiments with isolated intestinal segments in Tyrode solution, the question of digestive destruction may be dismissed. The second possibility has not been considered in most investigations of this kind. Yet, it seems to be obvious that serosa and mucosa may differ with respect to permeability and presence of enzymes which destroy or modify chemical substances applied to them. The sequence of action on contractile tissue and intramural ganglion cells is necessarily opposite in these two approaches, and different types of responses may be expected in suitably arranged experiments. The conditions are rather complicated because 1, circular and longitudinal muscle layers may be stimulated directly, but independently; 2, in addition each may be affected through ganglionic stimulation, and 3, they may influence each other mutually. This is in accord with the variegated and often inconstant results reported by many investigators. Only a few report observations of drug action by application to the inner surface of the intestine, as compared with that obtained by adding the drug to the bath in which the preparation was suspended. Haag and Kahlson (1), using the Trendelenburg method, studied the influence of acetylcholine on the peristaltic movements of the guinea pig's intestine. In one set of experiments they let the agent penetrate from the bath fluid into the intestinal lumen by prolonged diffusion, and then replaced the bath fluid with pure Tyrode solution. In another group of tests they used from the beginning, inside and outside, Tyrode solution containing acetylcholine, and, after a period of adjustment, replaced the bath fluid with pure Tyrode solution. In each case they observed really the effect of withdrawal of acetylcholine, not that of a positive action.

Feigen and Campbell (2) everted the intestinal strip and let histamine or acetylcholine act on the mucosal surface with the gut in an inside-out position. The gut did not respond to these stimulators in such conditions. It may be questioned whether the everted position of the intestinal segment did not have a fundamental influence on the behavior of the organ considering how easily a piece of intestine reacts with movements or spasms to the slightest handling. The kymographic records in their paper show that the gut was usually in a resting, i.e., non-oscillating condition and that only the tonic contraction immediately following the application of the drug was registered. In the case of a slow penetration of the drug, a different or delayed effect might be expected.

In this investigation a modification of the Trendelenburg method was used by which strips of guinea pig's or rabbit's intestine were subjected to the action of various physiological agents by alternate application to mucosa and serosa.

**GENERAL CONSIDERATIONS.** The technique employed excluded the possibility of exact quantitative comparisons. Intestinal segments are not sufficiently uniform in their behavior to permit more than general conclusions concerning stimulation or inhibition. The generally favored practice of using the same tissue repeatedly after washing out has not been followed in this work, although it would be feasible to adapt the equipment to that procedure. Washing is bound to remove some of the physiologic constituents of the intestinal tissue, which thereby loses some of its responsiveness. The material lost may be choline (3) or a precursor of acetylcholine (4), which according to Feldberg and Rosenfeld (5) is continuously formed in the intestinal ganglia, or electrolytes and enzymes. On the other hand, if physiologically active substances attach themselves to receptors in the cell, it will take more than a short washing to remove them. Cantoni and Eastman (6) have showed that histamine treatment in large doses, followed by washing, diminished the response to a standard new dose of histamine. There is reason enough for skepticism with reference to the use of tissue with a "history". Variability caused by pre-treatment may well exceed that occurring naturally when new pieces of tissue are used in each experiment.

Emphasis has been laid on the method of killing and time elapsed until the tissue is used, in order to obtain optimal and uniform activity. We have tried every method recommended without finding fundamental differences in the guts removed without delay. Preservation of reserve pieces of gut was tried in either ice cooled or aerated Tyrode solution of 37°. A relatively rapid decrease of the sensitivity to peristaltic stimulation during storage in warm Tyrode solution was observed by Haag and Kahlson (l. c.) and by Ambache (7) after cooling. In our experience, sensitivity decreases in either case, as is evidenced by the need of increased internal pressure to elicit peristaltic waves. Our final procedure was to use only intestinal preparations showing movements before treatment in accord with the majority of specimens.

The first strip taken out gave usually a satisfactory response. We had, however, one case of a rabbit that seemed exceptionally frightened before it was killed by bleeding. Not a single intestinal segment of this animal was responsive, which led us to assume that there had been a large secretion of epinephrine causing a persistent inhibition of the intestine. The method described in the following is the one used in most of the tests and considered the most practical.

**METHODS.** Guinea pigs and rabbits were used. Two-thirds of the standard dose of Nembutal (65 mgm. per 5 lbs.) was given intravenously followed by the last third about 5 minutes later. By dividing the dose, a longer survival of the animals seems to be obtained. Nembutal seldom produced complete anesthesia, but a whiff of ether was then sufficient to produce immediate loss of consciousness. Much ether must be avoided; the anesthesia, while complete, should not be very deep. If the animal showed signs of reappearing reflexes after 1 or 2 hours, an additional small dose of Nembutal was given. The abdomen was opened in the

midline and a large loop of the middle part of the ileum was gently pulled out. A segment, supplied by one branch of the vascular tree, was tied off, then the corresponding blood supply was ligated. The intestine was trimmed closely from the mesenterium, but not stripped, and cut close to the ligatures. After replacing the intestine, the abdomen was closed with clamps and the animal, wrapped in a towel, placed into an open incubator. As needed, additional segments were removed, which thus were kept with their blood supply until used. As long as the arterial blood remained bright red, the gut was found to be serviceable, but whenever the color turned to purple, indicating beginning respiratory failure, the sensitivity was no longer satisfactory. The removed gut was washed out with 37° Tyrode solution, using a syringe and applying gentle pressure from above down.

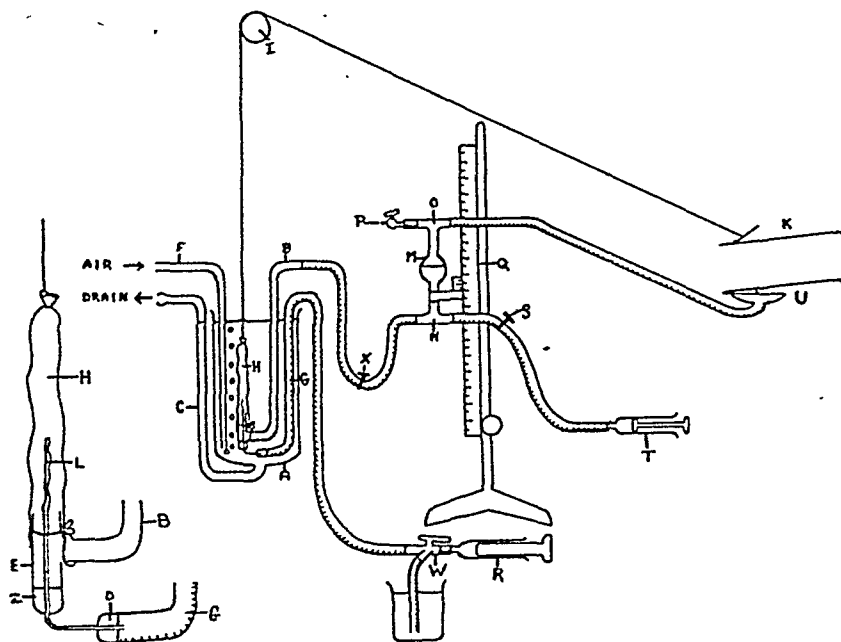


Fig. 1

*Apparatus.* Fig. 1'. The bath vessel, A, of a capacity of 250 cc., kept in water of 37.5°, contains Sollmann and Rademaekers' modification of Locke solution. The holding glass tube B ends in a short vertical tube E attached in T form (see insert): The cecal end of the gut strip H is tied firmly to the upper opening of E. The lower end of E is closed with a rubber cap Z through which passes a hypodermic needle (no. 22) D bent at a right angle and blunted at the end. It carries a piece of flexible capillary rubber tubing L of ca 2 cm. length. The hub of the needle is held in a piece of catheter tubing G that connects to the three-way stopcock W attached to syringe R and to a tube dipping in Tyrode solution. The holder tube B is connected by strong walled rubber tubing to the T-piece N and subsequently to the syringe T. S is a pinch-clamp. The other branch of N leads to a glass bulb M, held by the arm of a measuring support that permits the accurate adjustment of M to a variable height. The upper opening of M leads

to another T-piece *O*, carrying a stopcock *P* and connected on the other side to a tambour with writing pen *U*.

To attach the gut the whole portion of apparatus *BEG* is taken out and placed in a large Petri dish. As soon as the gut is tied to *E*, Tyrode solution is run from *T* and *M* until all air is removed. Then solution is pressed from *W-R* through *G* until this system also is free from air. (The capacity of tube *G* for liquid calculated from a marked spot close to the bend over the vessel edge to the end of the capillary tube must be known.) The upper end of the gut is now closed with a thread that runs over pulley *I* to the writing lever *K*. *M* is so filled with fluid that the surface is at the largest circumference of the bulb and at level with that in *A*, while *P* remains open. After about 20 minutes *M* is slowly raised, about 4 mm. at a time. As soon as peristaltic movements appear, as evidenced by oscillation of the liquid in *M*, *P* is closed. As a consequence, the tambour *U* begins writing. By this method, the liquid in *M* rises only insignificantly and the pressure of the water column remains practically unchanged with the peristalsis. The inertia of the system is also kept at a minimum.

In the records the upper line shows longitudinal changes, the lower, peristaltic movements. An upstroke corresponds to contractions. It is evident that the base line of the peristaltic movements may change, whenever the stopcock *P* has been opened. A shifting of the lower baseline, therefore, counts only if the stopcock has remained closed. Medication to the outside is added to the bath. To apply medication to the inside, the level of *M* is read, *P* opened and *M* lowered by a few millimeters. With a tuberculin syringe, the calculated quantity of the test material is injected into *G* at the marked spot and immediately washed from *R* into the gut with the necessary quantity of Tyrode solution. *M* is now adjusted to the previous level making allowance for the rise of liquid in *M*, and *P* is closed. The degree of dilution inside the gut is variable. It is estimated that after  $\frac{1}{2}$  cc. is injected, the substance may be diluted into 5 to 10 cc. If the liquid leaving *L* should run back into *E*, only the lower part of the gut would be exposed to medication. Peristalsis will gradually effect a mixing of the fluid within the gut and that in the tube *E*. Naturally, the effect must be gradual.

In these experiments the doses applied to the inside of the gut were kept smaller than those to the outside considering the rate of dilution of 1:10 average inside the gut as against 1:250 in the bath, but the reduction in dose was not remotely proportional, because it soon was found that the inside concentration had to be higher to be efficacious.

While stimulation still might be observed, even if only the lower part of the mucosa was bathed with the medication, the effect of a paralyzing drug might not be seen as long as the upper end of the gut was not exposed to it. For this reason, in the case of atropine, the following method was adopted: *P* was opened and *M* lowered to be below the surface in *A*. This drained the liquid out of the gut. Now the tube *B* was clamped at *X*, atropine was injected into *G* and washed into the gut and *M* raised again. After removal of the clamp *X*, *M* was adjusted to the previous level and *P* closed.

RESULTS. Spontaneous *longitudinal waves* were easily obtained in the rabbit gut but seldom in the guinea pig intestine. Internal pressure initiated *peristaltic*

waves, which often brought about pendular movements when these were missing. The following general observations were made: Longitudinal and peristaltic waves (in the following called l and p) are largely independent from each other and may run either in a continuous rhythm or in group waves with intermittent periods of rest. p waves may give a (mechanical?) starting stimulus to l in which case the contractions are synchronized. Single large contractions, especially of p, are called spikes. If p-spikes are of spastic nature, they cause lengthening of the gut and thus elicit a large l wave downward from the average tonus level (fig. 6 between 2 and 3).

*Tonus.* An increase in tonus of the longitudinal muscles (tl) is expressed by a shortening of the gut. If this increase is permanent it decreases or stops one or both movements by interference. If l movements persist at increased tl they usually coincide with a gradual reduction of tl. High tonus and wave movements are to some extent antagonistic. Absence of waves may be caused by relaxation (after atropine) or spastic tonus. This seems also to apply to the tonus of the circular muscles (tp). The stages of pendulum activity are represented by the pattern: 1, no movements by inertia; 2, waves in groups; 3, continuous waves; 4, arrhythmic and decreased waves with tonus increase; 5, stop by spastic contraction. A guinea pig's gut in stage 1 before treatment may be stimulated into activity by the same doses which in a gut in stage 3 cause inhibition by spasticity.

The following results must be appraised in the light of this interconnection of the various facets of intestinal movement.<sup>1</sup>

*Choline* applied to the outside of the rabbit's gut (5 to 10 mgm. in 250 cc.) stimulates tl *temporarily* [14, +14] and suppresses p [+9], later spastic p waves occur which are characteristic for choline [+10]. tl drops occasionally to levels below the initial [+3].

*Inside application.* (2-6 mgm.) tl is very slightly, or not at all, stimulated [8, +6]. Slight increase of l and slight inhibition of p.

Outside application (10 mgm.) following inside, increases tl only for a few seconds [8, +8]. l is stimulated and p shows spastic spikes. In the guinea pig's gut, the tl increase after outside application persists longer [8, +8].

*Acetylcholine* (a.c.). Guinea pig's [18] and rabbit's [10] gut show similar trends with a difference in degrees (figs. 2 to 6). Outside (5-10  $\gamma$ ). tl, and in most cases tp, increase; continued additions of a.c. produce corresponding increases in tonus; l and p are progressively inhibited [15, +14].

Inside (0.1-1  $\gamma$  and 2  $\gamma$  up respectively). No influence on tl; tp increase is dubious; p is first, later l stimulated [13, +11]. 10  $\gamma$  cause arrhythmic waves, ending in arrest, of p [4, +4].

Application to outside after inside: tl reacts strongly to first as to subsequent doses with an increase that subsides only slowly [13, +13]; tp increase lasts less; l shows the usual antagonism to tl.

*Carbamylcholine* (rabbit gut only) [3]. Inside: 1  $\gamma$  ineffective; 3  $\gamma$  increase

<sup>1</sup> This report does not correspond in every respect to the kymographic records shown, because it is a summary of a large number of observations. The first figure in brackets [ ] indicates the total number of tests, the + number those supporting the statements made.

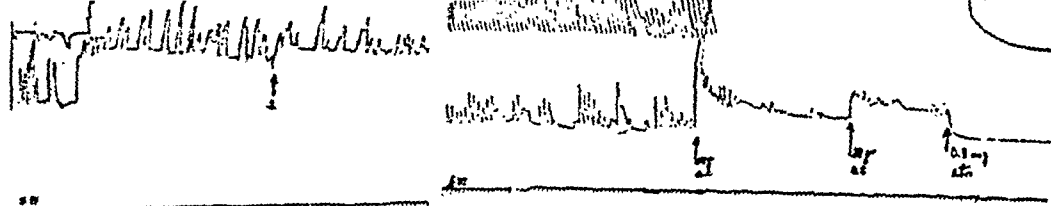


Fig. 2

Fig. 3

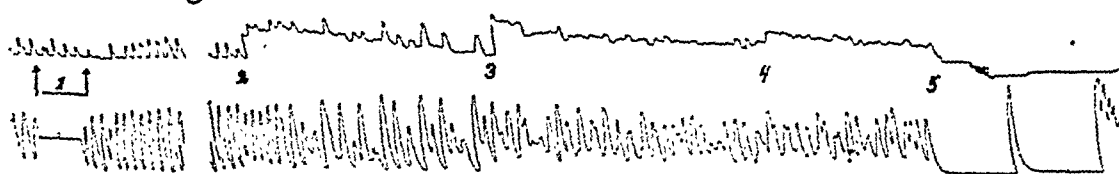


Fig. 4

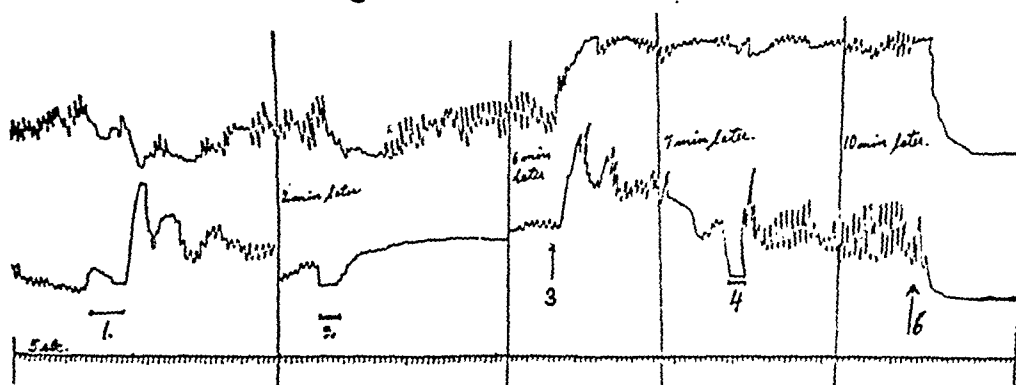


Fig. 5

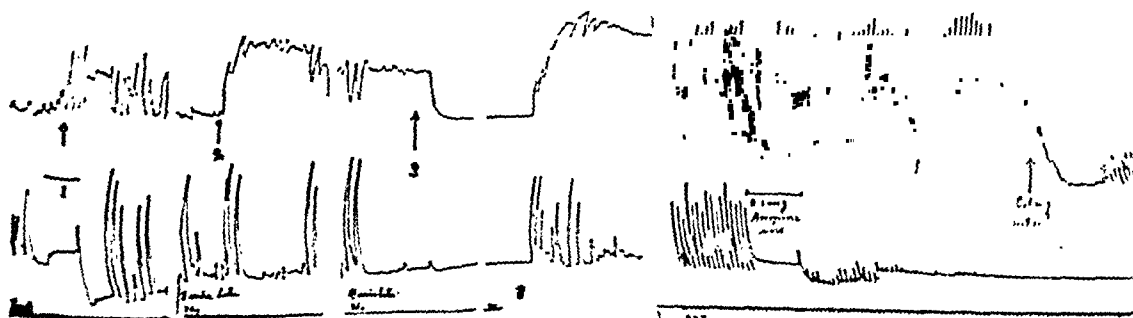


Fig. 6

Fig. 11

Time marking 5 seconds

Fig. 2. Rabbit gut. Acetylcholine applied to serosa. 1, 10 $\gamma$ . 2, 10 $\gamma$ .

Fig. 3. Rabbit gut. Acetylcholine and atropine to serosa as marked.

Fig. 4. Guinea pig gut. Acetylcholine. 1, 1 $\gamma$  to mucosa. 2, 5 $\gamma$  to serosa. 3 and 4, 10 $\gamma$  each to serosa. 5, 0.1 mgm. atropine sulfate to serosa.

Fig. 5. Rabbit gut. Acetylcholine. 1, 1 $\gamma$  to mucosa. 2, 2 $\gamma$  to mucosa. 3, 10 $\gamma$  to serosa. 4, 0.1 mgm. atropine to mucosa. 5, not shown, same as 4 with no change. 6, 0.2 mgm. atropine to serosa.

Fig. 6. Guinea pig gut. 1, 1 $\gamma$  Acetylcholine to mucosa. 2, 10 $\gamma$  Acetylcholine to serosa. 3, 0.1 mgm. atropine to serosa. (4-7 not shown: 200 $\gamma$  acetylcholine to serosa without effect, 1 mgm. acetylcholine causes slight contraction that is abolished again with another 0.1 mgm. of atropine.) 8, 10 $\gamma$  histamine to serosa.

Fig. 11. Rabbit gut, atropine first to mucosa, then to serosa.

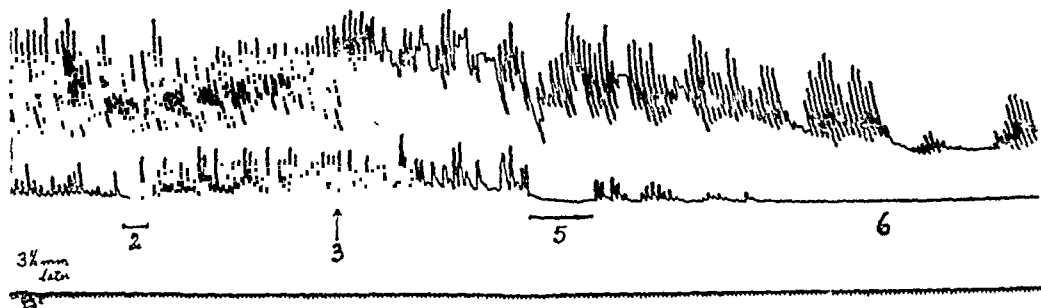


Fig. 7

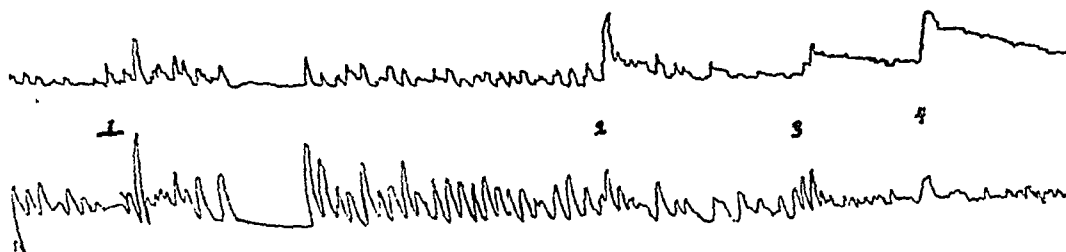


Fig. 8

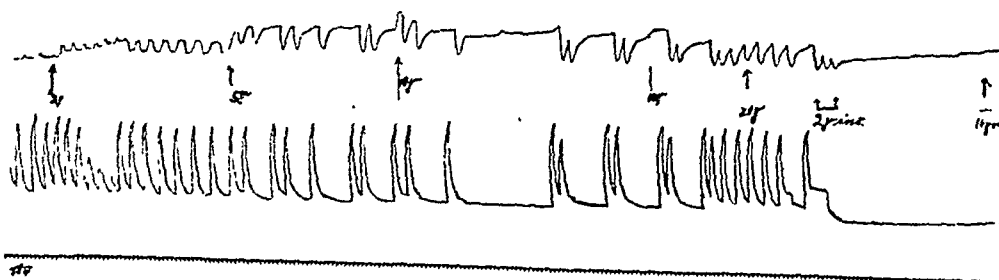


Fig. 9

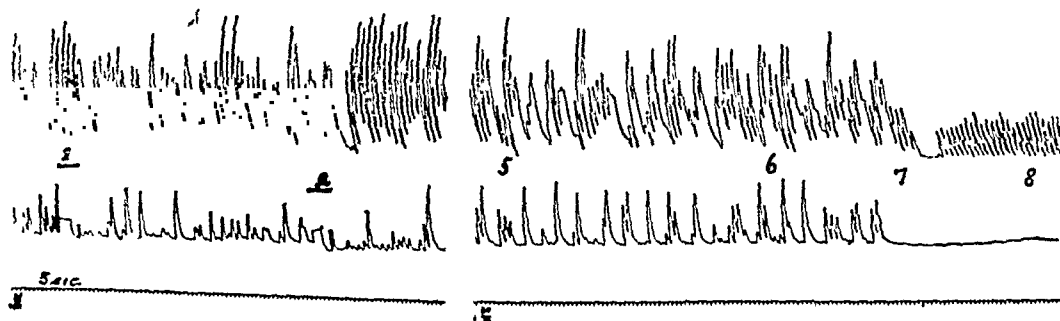


Fig. 10

Time marking 5 seconds

Fig. 7. Rabbit gut. 1, 2  $\gamma$  of acetyl methyl choline to mucosa (not shown) without effect. 2, 5  $\gamma$  same to mucosa. 3, 10  $\gamma$  of same to serosa. (4 omitted by error in marking.) 5, 0.1 mgm. atropine to mucosa. 6, 0.1 mgm. atropine to serosa.

Fig. 8. Guinea pig gut. Histamine-HCl. 1, 2  $\gamma$  to mucosa. 2, 5  $\gamma$  to serosa. 3, 10  $\gamma$  to serosa. 4, 20  $\gamma$  to serosa—note repetition of effect.

Fig. 9. Guinea pig gut continued treatment with histamine to serosa as indicated. (Note paralyzing effect of application to mucosa "2  $\gamma$  ins.")

Fig. 10. Guinea pig gut. Histamine. 1, 2  $\gamma$  to mucosa. 2, 10  $\gamma$  to mucosa. 3 and 4, 10 20  $\gamma$  to serosa (insignificant effect, not shown). 5, 100  $\gamma$  to serosa. 6, 500  $\gamma$  to serosa. 7, 0.2 mgm. atropine to serosa. 8, same as 7. Note absence of tonus increase.



tl *slightly*, increase p but not l. Outside: 10  $\gamma$  considerable and lasting increase of tl; partial inhibition of l and stimulation of p.

*Acetyl- $\beta$ -methyl choline* (Rabbit gut only) [3]. Figure 7 shows a typical response.

*Histamine*. The experience with histamine was more variable and contradictory than that with any other substance tested. The status of the intestine at the moment of treatment seems to be of fundamental influence on the direction of the response and on the dose required (figs. 8, 9, 10). Guinea pig, outside: 5-10  $\gamma$  causes uniformly a quick contraction (tl) [15,+15], which gradually subsides when l is abolished, faster when l persists. The interference with l and p is inconsistent; if the waves have been stopped, they reappear when tl drops. Successive addition of new doses causes renewed tl increase until finally refractoriness and relaxation may set in with absence of waves [5,+4].

Inside [10]. (2-10  $\gamma$ ) tp drops; l and p are first stimulated, but sudden periods of complete rest, lasting 1 to 2 minutes, appear (fig. 8) [10,+7]. 10  $\gamma$  may increase tl slightly, but after tl contraction by outside application, histamine from the inside causes relaxation [4,+2]. Contrariwise, when after high outside doses the gut is completely relaxed and at rest, 2  $\gamma$  inside may initiate new l and p, without return of tl [6,+3].

With series of outside doses started at subliminal quantities and gradually raised to 80-100  $\gamma$ , tl may remain unaffected while l and p increase [6,+3]. Additional 100  $\gamma$  then decrease l and p, but thereafter further doses are ineffectual. The tl contraction is more easily avoided by starting the series with 2  $\gamma$  to the mucosa (fig. 10) [3,+3]. The absence of tl increase is more frequent in the rabbit gut [4,+4]. Sudden gaps in movements following inside treatment are more seldom in the rabbit gut.

*Atropine*. *Outside*. One-tenth of one milligram in 250 cc. causes immediate maximal relaxation with cessation of all movements (fig 3.) [10,+10]. The same occurs if the strip has been treated before with a choline compound from the outside [30,+30]. The tl contraction induced by histamine is often reduced by atropine, but not abolished (fig. 6, no. 8) [8,+8]. The effect of atropine applied to the outside is changed if either atropine itself or a stimulating substance has before been applied to the inside.

*Inside* (fig.11). One-tenth of one milligram in a rabbit gut reduces and finally abolishes the p waves, while l waves continue unabated; tl is unchanged [8,+6]. Subsequent addition of 0.1 mgm. to the bath abolishes tl completely and both movements [8,+8], but l tends to start again with reduced strength [8,+5].

Following *acetylcholine* applied to the inside and outside, even massive doses of atropine outside change but do not stop p (fig. 4, no. 5) [10,+10]. A guinea pig's gut first contracted with a.c. outside, then relaxed with atropine outside, resumed p movements when a.c. was applied to the inside. This p movement was not interfered with by giving successively 0.025, 0.25 and 1 mgm. atropine to the inside and 0.5 mgm. to the outside. Atropine inside after a.c. to both sides is often without effect; paradoxically, it may stimulate p (fig. 4) or retard it. One tenth of one milligram given afterwards to the outside relaxes tl, as it always does, but may or may not interfere with p or l.

*Atropine, after treatment of both sides with choline.* The initial tl is not high; atropine inside causes further moderate relaxation and inhibits p [4,+4.] The effect on l is variable; it may increase, whereas it decreases if choline has been applied to the outside only. Atropine to the outside relaxes tl completely [8,+8], but l, and often p, may continue at a reduced amplitude.

*Atropine after carbamylcholine* [3]. (3  $\gamma$  to the inside, 10  $\gamma$  outside.) The gut is in a state of persistently high tl, strong p and irregular l. Atropine, 0.1 mgm. to the inside, leaves tl and l unchanged, inhibits p slightly. Outside application of 0.1 mgm. produces complete relaxation and rest.

*Atropine after acetylcholine* [3]. A typical response is shown in figure 7.

*Atropine and histamine* [30]. The antagonism of atropine to histamine, both applied to the outside, is relative, depending on the quantities of histamine used [10,+10]. If a gut has received 10–12  $\gamma$  histamine inside and then by repeated doses outside a total of 70  $\gamma$  or more, it usually shows a moderate tl, and active, but irregular l and p. Atropine (outside) causes now relaxation of tl, but l, or l and p, return after a short stop and show now a very regular movement (fig. 10, nos. 7–8). This is not abolished by more atropine. Atropine 0.1 mgm. inside after histamine inhibits p.

DISCUSSION. The data presented reveal as the most important facts: 1, The almost instantaneous contraction of the longitudinal muscles produced by choline and its esters entering through the serosa, and its counterpart, the equally prompt and complete relaxation by atropine. 2, The absence of these effects if the choline compounds or atropine are applied to the mucosa, and the need for an increase of concentration by 5–10 times, to obtain a response of any type by this route.

The first observation led Haag and Kahlson (1) to assume that the type of action of a.c. depends on the gradient of concentration and its direction, which theory has later been disproved by Voigt (8). Feigen and Campbell (2) are in agreement with our observations with reference to spastic contractions, but they failed to investigate peristalsis and pendulum movements. Our experiments prove considerable difference between the permeability of mucosa and serosa, but this difference is one of degrees and applies to atropine as well as to choline esters and histamine. The variegated and often contradictory responses are only partly open to interpretation; namely, on the basis of the present still defective knowledge of a.c. and atropine action. The following facts may be pointed out: Diffusion of a.c., being continuous, tends to establish a *permanently* raised local a.c. level at the myoneural junctions, which is fundamentally different from the temporary increases produced by nerve volleys. The effector cells, being subject to a continuous a.c. action, respond to it with a permanent tonus increase, that only wears off as the receptors of the muscle become adjusted to the sustained presence of a.c. This effects the tonic contraction of the longitudinal layer, and less noticeably, of the circular muscles also.

If the pendulum movements should be myogenic, the interfering influence of increased tonus may be explained by the fact that a contracted muscle has less freedom left for further contraction. However, a nervous influence is decidedly present in pendulum movements, as evidenced by the stimulation by small

quantities of a.c. and the inhibition by atropine. The a.c. reaches also the ganglionic synapses in Auerbach's plexus and it seems that here the first effect is stimulating, perhaps by supplying a subliminal quantity of a.c. so that the nerve ending's own a.c. production may cause higher temporary levels, whereas excessive quantities which flood the synapses with a.c. tend to minimize these same effects and cause inhibitions. For analogous reasons, nerve impulses arriving at the myoneural junction, when a certain a.c. concentration already prevails, cause proportionately less increase of same, namely, at the rate as the concentration increases, and finally are ineffectual. This gives an alternative explanation for the observed antagonism between tl and l.

The peristaltic waves which are elicited by a myenteric reflex brought about by the distention through the internal pressure can be influenced also by a.c. at both levels, the ganglionic and the myoneural. Thus, depending on conditions, stimulations or inhibitions appear. The fluctuations in concentration of a.c. are perhaps responsible for the change in reaction of circular muscle tonus to a.c. from the serosa, after a.c. has also been applied to the mucosa. In figures 2 and 3, when a.c. is applied to the outside only, tp shows an increase, which is absent in figures 4 and 6 when the mucosa also has been treated, and in figure 5 it appears but is far less sustained than with tl.

Atropine as a blocking agent to the myoneural junction is consistent in relaxing the tonus after application to the outside. The wave movements are stopped only in the untreated gut, or when choline esters have been applied to the outside. After inside treatment with a.c. and, which is more surprising, with atropine itself, atropine to the outside may not stop the wave movement. The cessation of p but not of l after application of atropine to the inside, suggests that the diffusion even after passing the mucosa, is slower in the direction mucosa  $\rightarrow$  serosa (figs. 11 and 7, no. 5). The paradoxical increase in p after inside application of atropine seen in figure 5, no. 4, can be explained as a consequence of removal of an interfering tonus. The same mechanism explains the regularizing influence of outside atropine on l in figure 10, no. 7. The inhibition of atropine on tonus, uniformly observed if it reaches the effector cells in sufficient concentration, is not always duplicated by the effect on wave movements, which may be decreased but not abolished, especially when choline esters have been applied from the inside. The action of atropine is not wholly explained as a block at the myoneural junction.

It is concluded that autonomic drugs, if efficacious orally, have not a direct influence on the intestine but are absorbed into the circulation and then return with the blood stream. The effect by diffusion through the serosa is unphysiological. Nutrient substances, oxygen, and drugs given parenterally, and probably orally, reach the intestinal wall normally by way of the blood.

Histamine appears to influence the muscle directly. The partial relaxing effect of atropine after histamine may be due to the removal of the neurogenic tonic effect, thus leaving the myogenic histaminic effect as the difference. This, however, does not explain why histamine, quite desultorily, produces relaxation (fig. 8, after 1). Barsoum and Gaddum (9) claimed that when histamine has once

acted on a tissue, a second histamine dose is without effect, which is true only in special and well defined conditions (fig. 10). An additive response is obtained instead with equal frequency (fig. 8). Histamine still remains a substance of puzzling physiological changeability.

#### SUMMARY

Trendelenburg's method of registering peristaltic and pendular movements of the isolated rabbit's and guinea pig's intestine has been modified, so that substances could be brought in contact with either the serosa or mucosa. The spastic effect of choline ester (choline, acetylcholine, carbamylcholine, acetyl- $\beta$ -methyl choline) and histamine on the tonus of the longitudinal muscles and the complete relaxation produced by atropine when added to the bath fluid, are not obtained when these substances are brought into the intestinal lumen, although at higher concentrations. The choline esters applied to the serosa tend to stimulate the pendulum and peristaltic movements, but the spastic tonus increase may interfere with both, preferentially the pendular movements. Choline esters applied to the mucosa tend to stimulate peristalsis first, secondarily the pendular waves; atropine passing through the mucosa causes a decrease of the peristaltic and less of the pendular movements. If atropine is applied to the serosa, after either atropine or choline esters have been introduced into the intestinal lumen, the tonus is abolished, but longitudinal waves alone or peristalsis also persist. The action of histamine is equally altered by application to the serosa; but this substance is quite variable in any application. The response of the intestine to autonomic agents depends largely on the initial tonus and motility of the strip used. The possible mechanism of some of the observed reactions of the intestine is discussed.

The observations suggest that autonomic drugs, if efficacious orally, act not locally on the intestine but are first absorbed into circulation and then return with the blood stream.

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# A STUDY OF CORONARY FLOW UNDER CONDITIONS OF HEMORRHAGIC HYPOTENSION AND SHOCK<sup>1</sup>

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Considerable evidence has accumulated indicating that a cardiac factor is at least partially responsible for the hemorrhagic shock syndrome. The reduction of cardiac output in hemorrhagic shock in spite of adequate central venous pressures has led to the suggestion that the contractile power of the heart is impaired (1). The failure of the heart to maintain cardiac output could be due either to a metabolic myocardial depression resulting from an inadequate coronary flow during the prolonged hypotensive period, or to an ischemic myocardial depression resulting from a failure to re-establish an adequate coronary flow following restoration of normal blood volume. Reduction of coronary flow has been alluded to at various times, but to our knowledge no previous investigators have attempted to estimate coronary flow during hemorrhagic hypotension and shock. A need for quantitative data seemed indicated, so this investigation was undertaken. The technical difficulties attending the estimation of coronary flow are numerous, but recent improvements in apparatus and techniques have made it feasible to follow sequential changes in coronary flow in protracted experiments.

**SPECIAL APPARATUS.** The coronary flow in the anterior ramus descendens was estimated by the use of an optically recording flow meter of the perfusion type in which the rate of pressure decline within the meter is nearly proportional to the rate of inflow into the perfused vascular bed. Since the mean coronary flow, and not phasic flow, was of primary interest, it was desired to use an instrument which was simple in principle and use, yet gave a fairly reliable indication of mean coronary flow. The flow meter used (fig. 1) is somewhat similar in construction to that described by Green and Gregg (2), but differs in the method of operation.

The cannula *A* was inserted into the anterior ramus descendens and the artery perfused with blood from the left subclavian artery through the side arm and tubing *B*. In preparation for taking a record of coronary inflow the reservoir *C* was filled to the same level preceding each record from the saline pressure line *D*. Turning the stopcock *F* connected the optical manometer *G* and flow meter to the Hg manometer *H*, the zero pressure of the latter having been set previously to an arbitrary blood pressure zero (level of the dog board). The pressure within the flow meter was raised to about 10 mm. Hg above mean arterial pressure or to a predetermined pressure by means of the aspirator bulb after which stopcocks *I* and *F* were closed. The flow beam projected from the optical manometer was placed on the extreme left side of the photokymograph

<sup>1</sup> Supported by a grant from the Commonwealth Fund.

lens, the mirror on the manometer being mounted so that the light beam traveled from left to right when the pressure within the flow meter decreased. The sensitivity of the optical manometer was adjusted so that the withdrawal of 1 cc. of fluid from the flow meter caused a deflection of approximately 3 cm. (camera distance 2 meters) corresponding to a pressure decline of 12-14 mm. Hg in the flow meter. The sensitivity could be adjusted by changing the air capacity

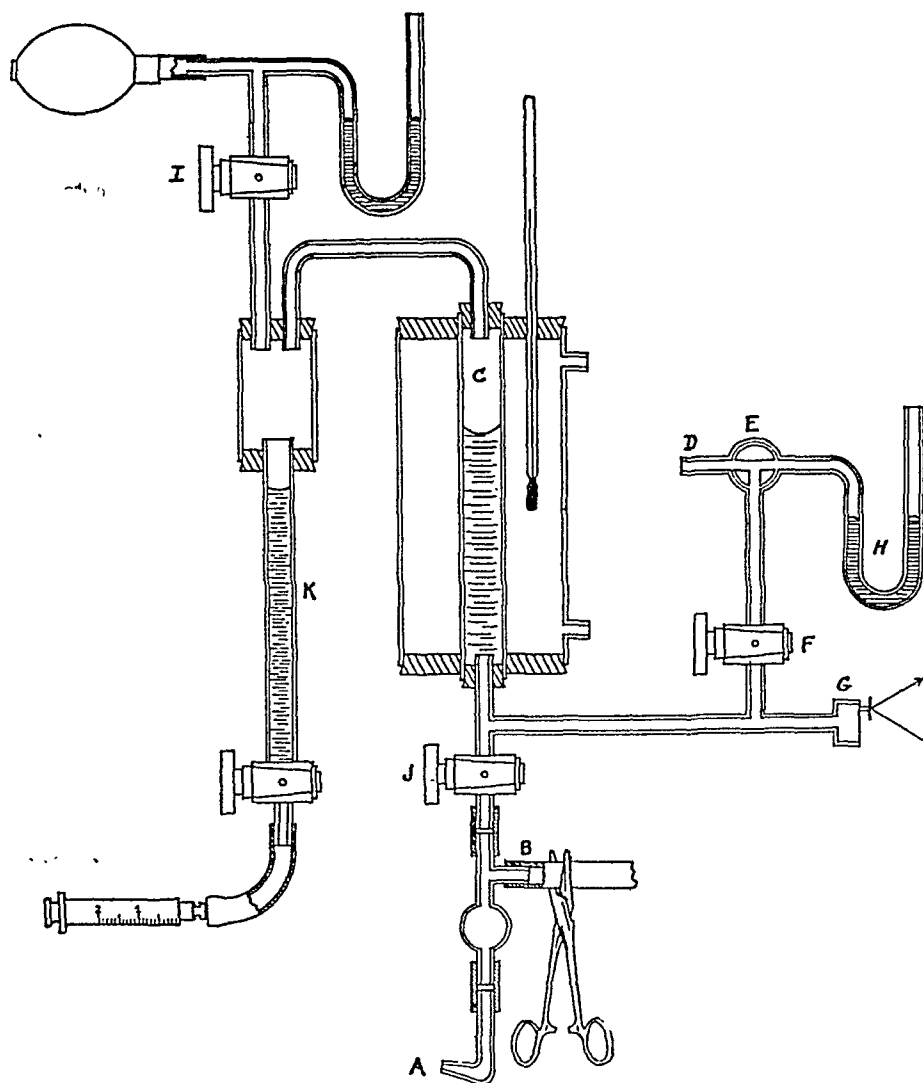


Fig. 1. Diagram of perfusion type flow meter. Described in text.

of the reservoir or the sensitivity of the manometer membrane. In order to take a flow record it was necessary only to clamp the tubing *B* and simultaneously open stopcock *J*. When the pressure in the flow meter declined below mean blood pressure stopcock *J* was closed and the clamp on the tubing released.

The flow meter was calibrated for each experiment by withdrawing 1 cc. of fluid at various pressures by means of a tuberculin syringe and constructing a calibration curve. Withdrawing fluid from the micropipette *K* allowed cali-

brations to be made during the course of the experiment. The relationship between pressure decline and outflow is not a linear one chiefly due to the fact that successive equal decrements of fluid volume reduces the air capacity within the meter proportionately less for each decrement because of the increase in the size of the air space. However, the departure from a linear relation is not large and would not introduce a significant error even if disregarded, which it was not. The coronary cannulas used in these experiments were tested and only those capable of delivering well over 100 cc. of blood per minute at 100 mm. Hg pressure were used. It was not anticipated on the basis of available data that flows greater than 100 cc. per minute would be encountered (3).

Viscosity changes due to the use of saline in the flow meter reservoir were avoided by introducing a bulb into the lower portion of the flow meter. The volume of blood in the lower portion of the flow meter (below stopcock *J*) amounted to 6–8 cc., whereas the volume of saline expressed from the reservoir during the course of taking a record never exceeded 3–4 cc. Thus, there was little mixing of blood and saline and the coronary bed supplied by the auto-perfusion received blood fresh from the subelavian artery during the taking of flow records. This scheme obviated three difficulties, *a*, the injurious effect of perfusing with saline, *b*, the viscosity change at the moment of taking a flow record, and, *c*, the settling out of red corpuscles when blood is introduced into the reservoir.

Changes in temperature of the perfusion blood due to contact with saline were minimized by having the reservoir enclosed in a glass jacket through which water from a constant temperature bath (38°C.) was circulated.

Two types of flow records can be obtained with this kind of flow meter. If flows are estimated at a pressure equal to the existing mean aortic pressure such flows indicate the magnitude of actual flow through the perfused coronary vessel at that particular pressure. These will be referred to as *actual coronary flows*. If an arbitrary perfusion pressure is chosen and estimations of flow are made throughout the experiment at such a pressure the flows so obtained in reality measure the resistance to flow encountered in the various phases of the experiment. We have designated these as *potential coronary flows*. The arbitrary perfusion pressure chosen in each experiment was a pressure equal to the control mean aortic pressure. Actual coronary flows were obtained in six experiments and potential flows in three. In three additional experiments both types of flow were obtained, the record of actual coronary flow being taken first and the potential flow record about two minutes later in each case.

**EXPERIMENTAL PROCEDURE.** Dogs weighing 10–22 kgm. were anesthetized with morphine sulphate and sodium barbital as previously described (4). Both femoral arteries and a femoral vein were cannulated for the purposes of bleeding, reinfusion and the continuous recording of mean arterial pressure with a Hg manometer. The right carotid artery was exposed and a tracheal cannula inserted. The chest was then opened wide by mid-line thoracotomy under adequate artificial respiration. Following the latter procedure, 440 units of heparin<sup>2</sup> per kgm.

<sup>2</sup> The heparin was generously supplied by Lederle Laboratories, Pearl River, N. Y., through the courtesy of Doctor Jukes.

were injected intravenously and a total dose of 550 units injected every 30 minutes thereafter. A cannula was introduced into the aorta or innominate artery via the right carotid artery for the optical recording of aortic pressure. A large glass cannula connected to about six inches of thick walled rubber tubing ( $\frac{3}{16}$  inch bore) and filled with heparinized saline was inserted into the left subclavian artery.

When the above preparations had been completed the heart was suspended in a pericardial cradle, care being taken not to interfere with venous return. A short section of the anterior ramus descendens was dissected free. Before beginning the dissection the coronary distribution was carefully examined and a site chosen which was relatively free of side branches and, peripheral to which, a considerable area of the myocardium was supplied by the ramus and its branches, it being desirable to perfuse as large a vascular bed as possible. Owing to the extreme variability of coronary distribution it was often necessary to compromise and choose a site which offered a reasonable chance for successful cannulation.

The lower section of the flow meter (below stopcock *J*) was detached and filled with heparinized saline, the upper end being closed with a glass plug. The tubing from the subclavian artery was connected to the side arm *B* and all bubbles worked into the upper end of the assembly. The coronary artery was then ligated and the cannula quickly inserted and tied into the artery peripheral to the ligature. Blood flow was established immediately, the whole operation taking somewhat less than 10 seconds. The cannula was aligned and fixed in such a manner that the movements of the heart did not mechanically occlude the flow during the cardiac or respiratory cycle. The dog board was moved under the flow meter and the cannula assembly connected to the major portion of the flow meter. The heart was covered with moist pads to prevent drying and heat was conserved by covering the thoracic field of operation with dry towel-ing and maintaining the rectal temperature by the application of external heat.

Before instituting the hemorrhagic shock producing procedure several records of control flow were taken at 5-10 minute intervals during temporary cessation of artificial respiration (as were all subsequent records). The method developed in this laboratory for producing hemorrhagic shock was used (5). The dogs were bled and the mean arterial pressure maintained ca 50 mm. Hg for 90 minutes after which the arterial pressure was lowered or allowed to decline to ca 30 mm. Hg for 45 minutes or until repeated small reinfusions failed to maintain the 30 mm. level. All withdrawn blood (warmed and filtered) was then reinfused and the subsequent course of events observed. Records of coronary flow were taken at frequent intervals throughout the experiment.

**ANALYSIS OF RECORDS.** Segments of a series of records from a typical experiment (expt. C) are reproduced in figure 2 which illustrate the method of calculating mean coronary flow. This was done as follows: Mean arterial pressure was determined by integration of the aortic pressure curve (AP) and checked against the femoral mean arterial pressure record. The point at which the pressure on the perfusion curve (PC) was equal to the mean aortic pressure was determined and a line tangent to the flow curve was drawn through this point



extending a distance equal to at least two seconds on the record. A perpendicular was dropped on a time line so that it intersected the tangent and a horizontal line was drawn so that it intersected the perpendicular on the left and the tangent on the right at a distance equal to two seconds from the perpendicular. This completed a triangle, the height of which indicated the rate of fall of pressure within the flow meter at that particular perfusion pressure. The

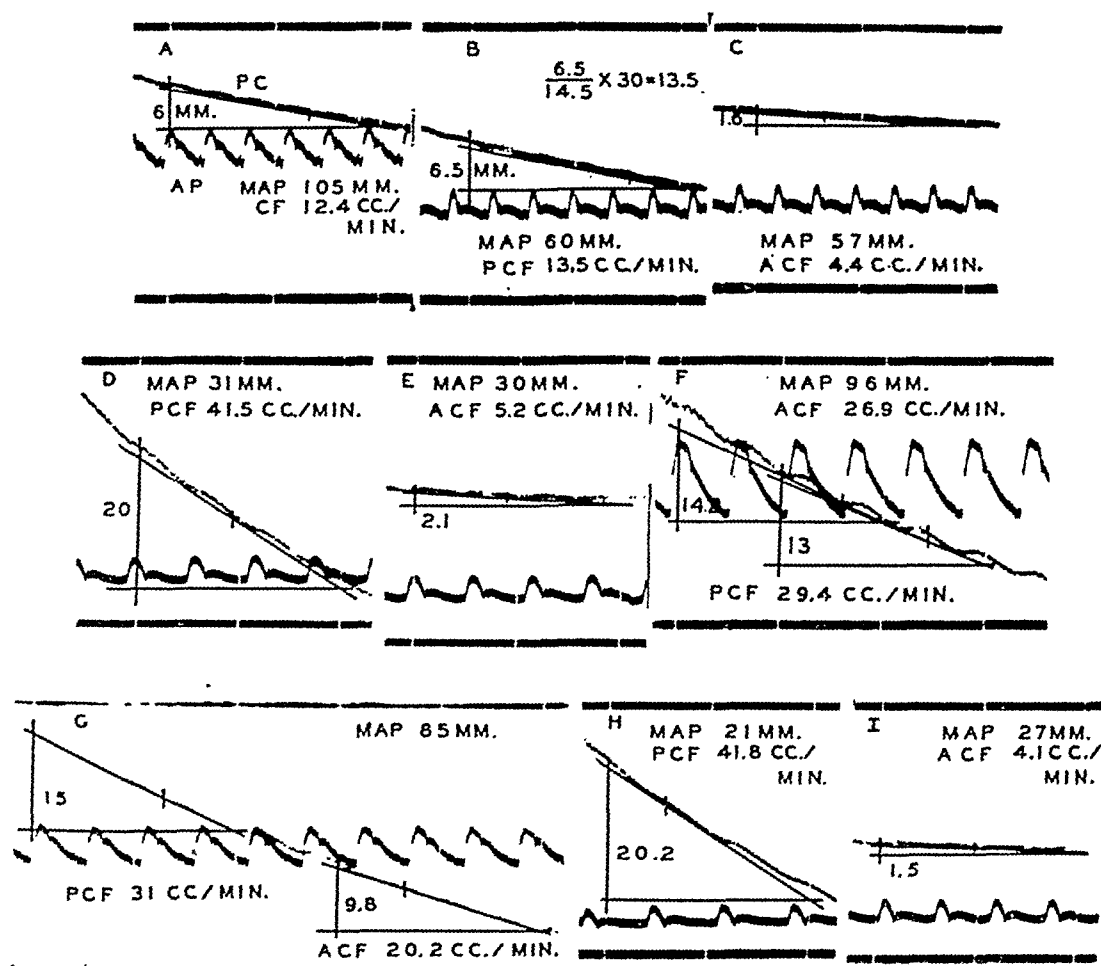


Fig. 2. Segments of optical records of coronary flow and aortic pressure from experiment C. Top, base line for coronary perfusion curve (PC); AP, aortic pressure curve; bottom, aortic pressure curve base line. Segment A, control; segments B and C, potential and actual coronary flow during early 50 mm. period; D and E, potential and actual coronary flows in late 30 mm. period; F, immediately after reinfusion; G, 15 min. after reinfusion; H and I, 95 min. after reinfusion. Discussion in text.

mean rate of coronary flow per minute was then calculated by multiplying the pressure decline by 30 and dividing by the decline in pressure caused by the withdrawal of 1 cc. of fluid from the flow meter at that particular perfusion pressure. Few artifacts were observed on the flow curves except some vibrations which occurred at the beginning and end of each record due to manipulation of the stopcock and clamp on the flow meter.

When records were taken at very low pressures (30 mm. period) the rate of

pressure decline was occasionally so slow that it became necessary to terminate the record as there was danger of asphyxial changes. As a consequence, in a few records the perfusion pressure never declined to mean aortic pressure level and calculations of coronary flow had to be made at perfusion pressures slightly higher than actual mean blood pressure. Thus, some of the flow figures during the 30 mm. period are greater than the actual flow.

Various tests devised to measure the accuracy of the calibration of the flow meter and the measurement of records have led us to the conclusion that the instrumental error probably does not exceed 5 per cent.

**RESULTS.** All methods of measuring coronary flow involve rather drastic surgical preparation incidental to the use of the physical appliances. It is obvious to anyone familiar with such experimentation that some dogs withstand extensive surgical operations better than others. As a consequence, the ability of the individual animal to withstand such procedures becomes a factor to be considered. Our experiments have been evaluated with this in mind.

Comparing certain reactions of our operated dogs with those of unoperated dogs subjected to the same hemorrhagic shock producing procedure appeared to be the best method of judging whether the operative procedure alone had materially affected the usual course of hemorrhagic shock. We have found the following observations to be the rule in our hemorrhagic shock experiments on unoperated dogs: 1. Control mean arterial pressures are usually in excess of 100 mm. Hg. 2. The bleeding volumes to 50 mm. Hg are around 4 per cent of the body weight. 3. Reinfusion of all withdrawn blood following prolonged hypotension usually restores mean arterial pressures to approximately control levels. 4. Following reinfusion the mean arterial pressures are maintained for periods of  $\frac{1}{2}$  to 2 hours before declining. Adopting these observations as criteria of the condition of our dogs we found that only three dogs in our series of 12 technically reliable experiments conform to all of the criteria. Therefore, our conclusions are based chiefly on data obtained from these three dogs with the thought that such results most nearly represent the probable changes in coronary flow of unoperated dogs subjected to hemorrhagic hypotension and shock. A detailed analysis of data from these three experiments is presented below with pertinent data from the remaining 9 experiments.

*Control data.* The paucity of quantitative coronary flow estimations obtained by reliable methods make it desirable to present our control data rather fully, although such data represent only the flow into a portion of the anterior descending ramification. The control flow data are summarized in table 1. These flows represent the estimates obtained just prior to bleeding the dogs and not average flows over long periods of time. Several determinations were made during a 20-30 minute control period in each experiment to check the constancy of the control flow, but aside from changes in flow due to minor fluctuations in aortic pressure, the several control flows were in excellent agreement. In addition, experiment 0-117 (table 1) was in the nature of a control experiment, flows being estimated at intervals over a period of four hours. Although aortic pressure improved, the coronary flow remained surprisingly constant. Variations in the

size of the hearts, area of vascular bed perfused (as determined by site of cannulation), and the varying control aortic pressures preclude comparisons between actual volumes of flow obtained in different experiments.

*Experiment A.* The reaction of this dog in respect to coronary flow is plotted in figure 3. Immediately following withdrawal of blood to the extent of 3.3 per cent of body weight (a) the actual coronary flow decreased to 51 per cent of the control flow (18.7 to 9.5 cc./min.). Further bleeding (total hemor-

TABLE 1

*Control data on mean aortic pressure and coronary flow in a portion of the ramus anterior descendens, together with bleeding volume to 50 mm. Hg. Discussion in text*

EXPT. NO.	BODY WT.	MEAN AORTIC PRESSURE	CONTROL CORONARY FLOW	BLEEDING VOLUME TO 50 MM. HG
	kgm.	mm. Hg	cc./min.	cc./kgm.
0-119 (Expt. B)	15.4	145	26.7	44.0
0-123	13.0	140	66.5*	24.6
0-115 (Expt. A)	17.0	138	18.7	44.7
0-114	14.5	135	26.6	30.4
0-112	13.5	130	28.7	24.0
0-118	15	115	21.6	27.3
0-126 (Expt. C)	10.5	105	12.4	30.0
0-108	12	91	18.4	35.4
0-121	10.5	90	18.0	29.8
0-122	13	90	16.4	
0-116	13.5	90	13.6	27.4
0-125	15.5	85	8.3	23.8

EXPT. NO.	BODY WT.	TIME	MEAN AORTIC PRESSURE	CONTROL CORONARY FLOW
	kgm.	min.	mm. Hg	cc./min.
0-117†	11.5	0	84	9.1
		92	80	11.5
		191	83	10.8
		225	90	12.5
		250	102	11.8

\* No technical basis for exclusion

† Control experiment

rhage = 4.5 per cent of body weight) stabilized mean aortic pressure between 50-55 mm. Hg. During most of this period (a-b) coronary flow only reflected minor fluctuations in aortic pressure, but toward the end of the 50 mm. period while aortic pressure was stabilized at 50 mm. Hg a slight but definite increase (53 per cent to 62 per cent) in coronary flow was observed. At this time there was no change in heart rate. Such evidence of a decreasing resistance to flow at this point did not occur in every experiment but was frequently observed.

Reduction of mean aortic pressure to 35 mm. Hg (b) caused a further decrease to 31 per cent of control flow (6.7 cc./min.). The flow was stable throughout the 30 mm. period (b-c) with no tendency toward improvement being noted. Reinfusion of all withdrawn blood immediately restored mean aortic pressure to 83 per cent of the control pressure (c-d). Coronary flow increased from 6.0 cc./min. before reinfusion to 47.0 cc./min. (32 per cent to 250 per cent) a few minutes after completion of reinfusion. Such tremendous increases in flow were observed in every experiment where reinfusion effectively increased mean aortic pressure. In one experiment (not plotted) the coronary flow was 60.5 cc./min., or 445 per cent of the control flow immediately after reinfusion. Considering that the ramus anterior descendens carries somewhat less than 30 per cent of the total coronary flow (3) the minute volume of flow is considerable in such cases. Flows such as these can mean only that resistance to coronary flow is very low following a prolonged period of hypotension.

Actual coronary flow declined from 250 per cent of control to 156 per cent over a period of 16 minutes during which time the mean aortic pressure was increasing from 115 to 135 mm. Hg (d-e), most of the decrease in flow occurring while the aortic pressure was stabilized at 135 mm. Hg. Obviously, the resistance to flow was increasing. As mean aortic pressure began to decline (e-f), marking the beginning of the circulatory failure (shock) the actual coronary flow also decreased. However, at a point some 173 minutes after reinfusion, the coronary flow again increased from 92 per cent to 167 per cent in spite of the falling mean aortic pressure (f-f'). A situation comparable to this late increase in actual flow was not observed in any of the other experiments and its true cause is uncertain. It might be pointed out that anoxia or hypercapnia results in increased coronary flow (6) and that this experiment was the most successful of our series from the standpoint of survival time. There is a possibility that the long continued artificial respiration (7 hrs.) produced an early stage of pulmonary edema and hence some degree of anoxia. This suggestion is supported by the fact that the aortic pressure and coronary flow fell rapidly and the dog expired 22 minutes after the peak of the rise (f'-g). Autopsy revealed the hemorrhagic duodenum characteristic of shock in dogs and the lungs were found to be hyperemic.

*Experiment B.* Changes in resistance to coronary flow were followed in some cases by estimating the flow at a constant perfusion pressure during the various phases of the experiment. The perfusion pressure selected was usually equal to the control mean aortic pressure and the flows obtained designated as *potential coronary flows* because they represent the flow that would have obtained if mean aortic pressure had been equal to perfusion pressure. A plot of such an experiment (expt. B) is shown in figure 3.

At the conclusion of the initial hemorrhage (a) the potential coronary flow had already increased to 193 per cent of the control flow (control flow = 26.7 cc./min.). In six experiments this was a consistent finding, the increases in potential flow ranging from 136 to 197 per cent. The potential flow in experiment B continued to increase to a maximum late in the 50 mm. period. The fluctuation

in flow noted on the ascending limb (a-a') was associated with an increase in mean aortic pressure which necessitated a substantial withdrawal of blood in order to stabilize the mean aortic pressure at 55 mm. Hg.

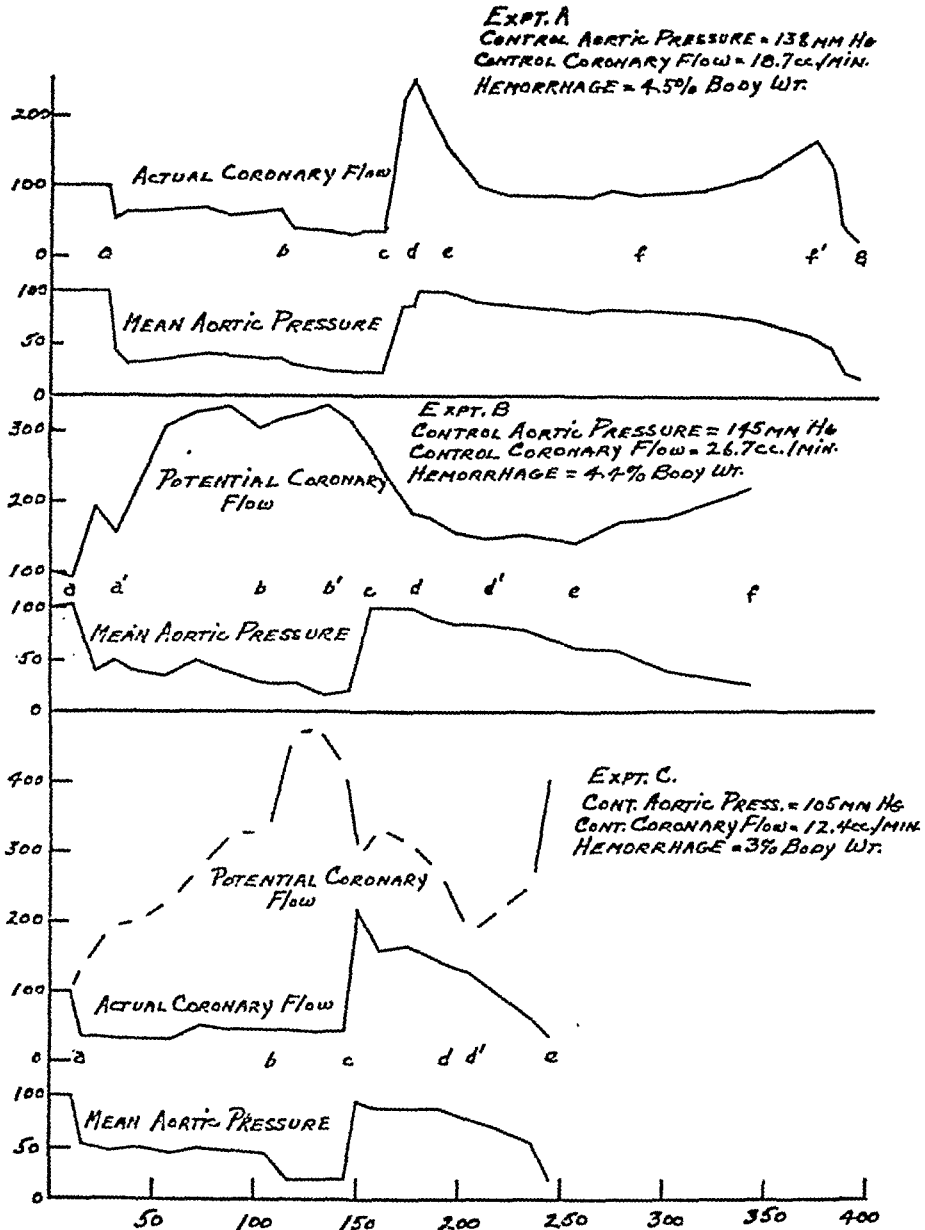


Fig. 3. Plots of three experiments showing sequential changes in actual and potential coronary flow during hemorrhagic hypotension and shock. The data are plotted as per cent change from control value (control = 100 per cent). Flow in cc./min. or aortic pressure in mm. Hg may be obtained by multiplying the per cent change by control figure. Ab-scissal time in minutes. Discussion in text.

Lowering the mean aortic pressure to 30 mm. Hg at *b* decreased the potential flow from 333 per cent to 304 per cent of control flow, but the decrease was only temporary, the maximum (333 per cent) being attained again late in the 30 mm.

period ( $b-b'$ ). A slight but definite decrease in potential flow was noted at the very end of the 30 mm. period (333 per cent to 311 per cent) which was not associated with any appreciable change in mean aortic pressure.

The reinfusion of all withdrawn blood ( $c$ ) restored mean aortic pressure to 97 per cent of control (140 mm. Hg) and caused a decrease of potential flow from 311 per cent of control flow to 275 per cent. This immediate decrease of potential flow immediately after reinfusion was observed in five out of six experiments. A satisfactory recovery of mean aortic pressure was not obtained in the nonconforming experiment (61 per cent of control pressure).

Potential flows continued to decrease after reinfusion, the flows being reduced from 275 per cent at  $c$  to 181 per cent at  $d$ , the mean aortic pressure remaining constant at 140 mm. Hg. At  $d$  aortic pressure began to decline progressively and potential flow likewise continued to decrease for a time ( $d-d'$ ). However, the flow became stabilized for a time ( $d'-e$ ) in spite of the falling aortic pressure, and then exhibited a progressive increase until the termination of the experiment ( $e-f$ ). The volume of potential flow was never reduced to the control flow following reinfusion, the closest approach being to 142 per cent of the control flow. Perusal of the data shows this to be true of all experiments in which potential flow changes were followed. The directional changes in potential flow as seen in this experiment are qualitatively typical of those observed in the other experiments, particularly the changes of flow in the immediate post-reinfusion and later shock phase of the experiments.

*Experiment C.* Both actual coronary flow and potential coronary flow were estimated in three experiments, one of which is plotted in figure 3. The actual coronary flow curve resembles that seen in experiment A with a few variations. In contrast to experiment A, when mean aortic pressure was lowered to 30 mm. Hg ( $b$ ) the actual coronary flow was not diminished ( $b-c$ ), indicating that a compensatory reduction in flow resistance had occurred. That this was the case is established by the fact that potential coronary flow increased from 324 per cent to 470 per cent of control (32.1 cc./min. to 47 cc./min.) during the same interval. Presumably then, the reduction of actual coronary flow observed in experiment A during the 30 mm. period occurred because flow resistance was already minimal and, if an actual coronary flow curve had been obtained in experiment B, it would have resembled that of experiment A rather than that of experiment C.

Actual coronary flow was greatly increased following reinfusion, the volume of flow being increased from 5.2 cc./min. to 26.9 cc./min. A 40 minute period of stabilized mean aortic pressure followed reinfusion ( $c-d$ ), during which time the actual coronary flow first decreased sharply and then more slowly. The heart rate accelerated slightly during this period (102 to 119 beats per min.). From  $d$  to  $e$  the mean aortic pressure and actual coronary flow declined rather quickly, the dog expiring 100 minutes after reinfusion. Due to the precipitous development of circulatory failure a post-reinfusion period of stabilized coronary flow did not appear. In this respect, experiment C more closely resembled the majority of the experiments than did experiment A. \*

Potential coronary flow increased progressively until late in the 30 mm. period in contrast to experiment B in which the maximum occurred late in the 50 mm. period. The decrease in flow immediately after reinfusion was well marked in this experiment (420 per cent to 296 per cent), as was the continued decline (to 272 per cent), while mean aortic pressure held constant (*c-d*). A decline of potential flow accompanied a decline of mean aortic pressure for a short time (*d-d'*), but then potential flow began to increase sharply (*d'-e*), although mean aortic pressure was well above shock levels. During the late increase in potential flow, both in experiments B and C, the heart rates were essentially constant except for the terminal records.

**DISCUSSION.** In view of evidence that a cardiac factor is involved in the genesis of hemorrhagic shock, it was of interest to us to ascertain whether the basis of myocardial damage and circulatory failure could be an inadequate coronary blood supply following reinfusion after a period of prolonged hypotension. There can be no doubt that during the period of hypotension the actual coronary flow is seriously curtailed, providing ample opportunity for either metabolic or hemodynamic disturbances, the effect of which might become apparent when the work of the heart is increased. The question of what constitutes an adequate coronary flow is a difficult one, since an answer requires a quantitative knowledge of the metabolic needs of the heart during various degrees of energy expenditure. Even if we had a thorough knowledge of the work output of the heart during the different phases of these experiments, the knowledge of the correlated quantitative need of oxygen, lactic acid, amino acids, etc., would be sadly lacking. Therefore, it is necessary to confine ourselves to a comparison between actual coronary flows in the non-shock state (i.e., early 50 mm. period) and in the shock state at equivalent aortic pressures.

It is evident that immediately following reinfusion, and for some time after, the coronary flow is adequate in the sense that it is greater than control flow in spite of the fact that usually mean aortic pressure is slightly less than control pressure. Flow comparisons at lower aortic pressures, such as can be made in experiment C, show that actual coronary flows during the shock phase of these experiments are at least equal to and usually greater than coronary flows at equivalent aortic pressures in the pre-shock state. For example, in experiment C during the early 50 mm. period mean aortic pressure was 57 mm. Hg and the actual coronary flow 35 per cent of the control flow. In the same experiment during circulatory failure the mean aortic pressure was 56 mm. Hg, but actual coronary flow was 59 per cent of the control flow. This observation can be made almost without exception at any level of aortic pressure in any of the experiments. We conclude, then, that there are no mechanisms operating which tend to reduce actual coronary flow other than the loss of pressure head due to the decline of mean aortic pressure. The data derived from potential coronary flow estimations indicate that resistance to flow in the shock state is always less than control, thus providing a foundation for the observation that actual coronary flows are somewhat greater during shock than one would expect on the basis of simple decline in aortic pressure.

That an inadequate coronary flow is not responsible for the circulatory failure following transfusion is further indicated by the fact that in most cases mean aortic pressure has already declined markedly before actual coronary flow is reduced to less than the control flow. This point is particularly well illustrated by experiment C. At the time actual coronary flow is reduced to 100 per cent (equal to control flow) the mean aortic pressure had already declined from 97 per cent to less than 75 per cent of control aortic pressure. This is true to a greater or lesser extent in all our experiments.

It would appear that the evidence for myocardial damage in shock must have its basis in a direct metabolic disturbance in the cells constituting the myocardium resulting from the reduced coronary flow during the hemorrhagic hypotensive period.

In view of the large changes in potential coronary flow encountered in this investigation the changes in resistance to flow merit some discussion. Unfortunately, the effects of extra-vascular support or compression, vasomotion, and changes in heart rate on coronary flow are not amenable to easy differentiation (7). However, several pertinent observations can be made on the basis of our data. In experiment A a short time after reinfusion actual coronary flow decreased from 225 per cent to 156 per cent of control flow, while the mean aortic pressure was constant at 135 mm. Hg, and the heart rate increased only from 150 to 153 beats per minute. Assuming that the constant aortic pressure indicates no change in the mechanics of ventricular filling and emptying the extravascular compression effect must have been reasonably constant and, since the heart rate change was negligible, the decrease in flow must have been due to active decrease in the diameters of the coronary vessels. A similar situation can be seen in experiments B and C.

The progressive increases in potential coronary flow during the late post-reinfusion periods of experiments B and C do not lend themselves to such an analysis because, although heart rates were essentially constant, a declining aortic pressure indicates a lessened mechanical compression effect and, hence, less resistance to flow, thus obscuring any vasomotor change. However, the great increase in potential flow during the 50 and 30 mm. periods of experiments B and C affords some evidence for an active vasodilatation. Aortic pressures were fairly stable in both experiments, the slight fluctuations which did occur not accounting for the magnitude of the changes in flow. In one case, experiment B, the heart rate increased from 150 to 162 beats per minute, while in experiment C, the heart rate decreased from 160 to 128 beats per minute. Apparently, heart rate change is a negligible factor because both flows were increased to well over 300 per cent of the control flow. It would appear that active increase in the diameter of the coronary vessels accounted for a great part of the decrease in resistance to flow. We tentatively conclude, pending clarification of the magnitude of the effects of changing ventricular mechanics and heart rate changes on coronary flow that, 1, during hypotension there is a vasodilatation in addition to a lessened mechanical compression effect which reduces resistance to flow, and, 2, that following reinfusion there is, in addition to an increased me-



chanical effect, a transient increase in resistance due primarily to active vasomotion. However, this soon gives way to a further reduction in flow resistance due to a decreased mechanical compression effect and perhaps in part to active vasodilatation.

In connection with the discussion of the coronary flow resistance it should be noted that the coronary system is the first vascular circuit to be investigated which exhibits a consistent decreased resistance to flow during hemorrhagic hypotension and shock. While estimations of total peripheral resistance indicate that TPR is extremely variable but generally increased under these conditions (8,9), flow resistance is consistently increased in the leg (10), kidney (11), and spleen (12). In view of these observations, it would appear that flow resistance in the coronary system reacts in a manner opposite to that of some of the other vascular circuits. To what extent a decrease in coronary flow resistance would affect TPR is problematical, but it is clear that not all vascular circuits contribute to the increased TPR sometimes observed during hemorrhagic hypotension and shock. Since the completion of these experiments Selkurt (13) has obtained evidence of a reduced resistance to blood flow in the gut under somewhat similar conditions. The existence of varying directional changes in flow resistance in different vascular circuits during hemorrhagic hypotension and shock provides a basis for a redistribution of blood flow which may be of some importance in explaining circulatory failure in shock.

#### SUMMARY

Changes in coronary flow and flow resistance were estimated during standardized hemorrhagic hypotension and shock in dogs by use of a perfusion type flow meter. Coronary flow decreased to 30–60 per cent of control flow during the 50 and 30 mm. hypotensive periods and flow resistance was greatly decreased. Following reinfusion of all withdrawn blood and restoration of mean aortic pressure to control level the coronary flow increased to 121–420 per cent of control flow. The augmented coronary flow was maintained until some time after circulatory failure had intervened and almost invariably was greater than the coronary flow at equivalent aortic pressure in the pre-shock state. Flow resistance during circulatory failure was always less than control resistance. Vasodilatation appeared to play a prominent part in the decrease in resistance.

It is concluded that circulatory failure following hemorrhagic hypotension is not precipitated by an inadequate coronary flow following restoration of aortic pressure by reinfusion.

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## PHASIC CHANGES IN INFERIOR CAVA FLOW OF INTRAVASCULAR ORIGIN<sup>1</sup>

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The influence of cardiac action and respiratory movements on the rate of blood flow in the inferior vena cava have been frequently discussed with somewhat contradictory conclusions. (For reviews see Gollwitzer-Meier (1), and Franklin (2).) Since a number of extravascular variables influence cardiac and respiratory variations in venous return it seemed desirable to restudy the changes due to intravascular forces uninfluenced by extraneous effects. This can be done in dogs with an open chest when they are placed in such a position that movements of the heart neither impinge directly on the inferior vena cava nor exert traction upon it. Registration of phasic changes in flow was accomplished by modified Pitot tube cannula which, after insertion into the inferior cava, was held in place by snugly fitting rubber ring washers. The instrument illustrated in figure 1 was connected by lead tubes to a sensitive differential manometer described by Gregg and Green (3). The construction of the Pitot cannula is the essential feature of the whole apparatus. As is well known, the small differences of up- and down-stream pressures in veins make it difficult to register venous flows by differential pressure manometers. Trials with artificial streams having flow rates and pressure gradients similar to those of the inferior vena cava revealed that greater differentials could be obtained if the tube on the afferent side was provided with a small baffle plate (fig.1-A), and the tube on the efferent side (M) was placed so as to transmit the pressure at the point of maximum blood velocity. Such dissimilar construction of the Pitot tubes was allowable only because early attempts to measure vena cava flows with the conventional Pitot tubes showed that backflows if existent were minimal. The position of the double tube could be adjusted by washers placed in the connection shown at *x* until it did not interfere with natural flow as detected by a rise of pressure in the abdominal inferior cava. After each record a zero flow line was photographed in relation to a base line, and at the termination of an experiment deflections of the light beam during various determined rates of flow were recorded. Plots of these deflections against various flow rates on log paper gave a straight line curve which served as a calibration scale for records.

**PROCEDURE.** The surgical procedures included the following sequential steps: 1. The dog in a supine position was rotated toward the left, so that the heart was naturally displaced toward the left of the thorax. 2. A long incision was made through the 5th intercostal space on the right side by means of a thermocautery and the ribs were separated widely by a special retractor.

<sup>1</sup> This research was supported by a grant from the Commonwealth Fund.

3. After attention to hemostasis a dose of 4 mgm./kilo heparin<sup>2</sup> was injected intravenously, and doses of 0.5 mgm. were repeated every 30 minutes. 4. Arterial blood pressures were recorded from a carotid artery by means of an optical pressure manometer, calibrated quantitatively. 5. A pneumograph placed about the abdomen and connected to an optical pressure manometer measured diaphragmatic movements.

The technique of inserting the Pitot cannula consisted of the following steps:

1. The inferior vena cava was freed from the phrenic nerve and adjacent tissue, clamped as high and low as possible, and cut completely.
2. A rubber washer to hold the cannula was quickly slipped over each end of the cut cava.
3. The cut edge of the lower segment was grasped by four hemostats, the can-

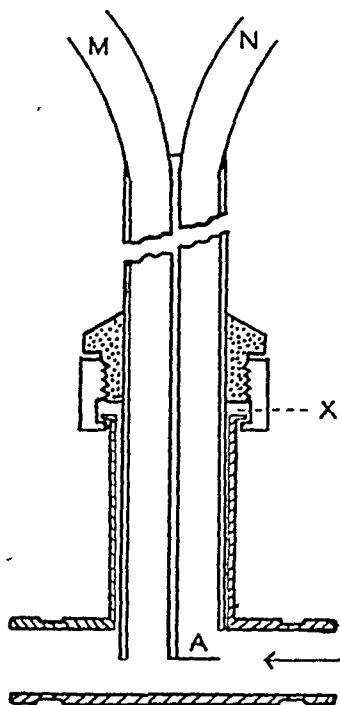


Fig. 1. Diagram of Pitot tube cannula inserted into the inferior vena cava and connected at M and N with a differential optical manometer to record changes of flow rates in the inferior vena cava. Description in text.

nula attached to the differential manometer was inserted and fastened by gently shoving the washer into a groove (fig.1). 4. After filling the cannula with saline solution from the manometer tubes the other end of the cannula was similarly inserted into the central stump of the cava and fastened by its rubber washer. 5. Both of the cava clamps were removed and the natural flow of blood resumed. The entire procedure required 3-5 minutes, during which time recorded femoral arterial pressures fell progressively to circa 30 mm. Hg, but after release returned to normal levels. No evidence was found that this brief period of drastic hypotension and venous stasis influenced the subsequent dynamic course of events.

<sup>2</sup> We are indebted to Lederle Laboratories, Inc., Pearl River, N. Y., for the heparin used in these experiments.

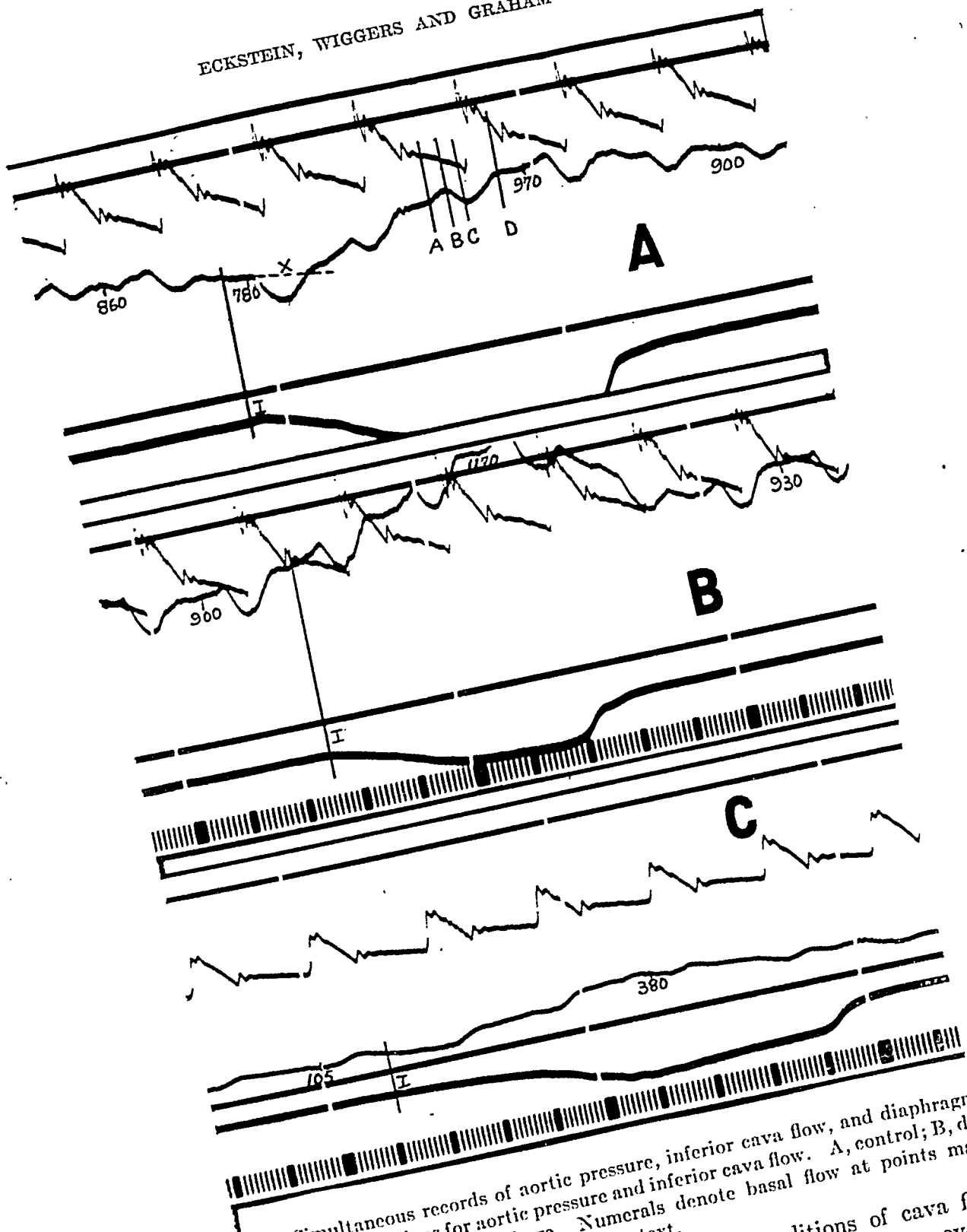


Fig. 2. Simultaneous records of aortic pressure, inferior cava flow, and diaphragmatic movement, with base lines for aortic pressure and inferior cava flow. A, control; B, during saline infusion; C, after hemorrhage. Numerals denote basal flow at points marked. Time in 0.02 and 0.002 second. Discussion in text.

**RESULTS.** The phasic changes under different conditions of cava flow in relation to central arterial pressure curves and natural respiratory movements are illustrated in figure 2. The animals were maintained under mild but ade-

quate artificial respiration except during brief periods when such records were taken. As illustrated by the lower curves, slow spontaneous respiratory movements reappeared, of which descent of the diaphragm and lower ribs alone is of importance for our studies.

Figure 2-A shows the phasic cardiac and respiratory variations in flow under conditions usually resulting soon after insertion of the cannula. By application of calibration charts the cardiac variations could be determined. Their time relations show the following sequential changes labeled in one cycle; A-B, a slight acceleration in flow, probably coincident with rapid ventricular filling from the atria; B-C, a marked retardation during the first half of atrial systole, C-D, a sharp increase during ventricular systole, followed (after D) by a fairly stable flow until ventricular filling starts again. This stretch of stable flow would appear to be the best point for measurement of basic flow changes under different dynamic conditions. As regards interpretation, it becomes evident that these cardiac variations of flow are determined fundamentally by changes in atrial pressures during the cardiac cycle. Since a short period of apnea intervened between respiratory movements the effects of diaphragmatic descent and ascent on venous flow can be compared with the natural rate of flow due to cardiac action alone.

As illustrated at I, the first effect of diaphragmatic descent consists of a slight reduction in the basic flow curve (dotted line), as a result of which an atrial systole coinciding with this drop causes an apparently greater drop in pressure. Following this minor temporary reduction the basic flow increases significantly, in this case from 780 to 970 cc. per minute. The cardiac variations continue unchanged in design. Obviously, the diaphragmatic contraction materially increases the flow due to augmentation of intra-abdominal pressure and/or compression of the liver. Similar results in 9 dogs warrant the conclusion that, except for a slight degree at the beginning of inspiration, no support was found for the belief that descent of the diaphragm increases caval resistance and reduces flow by muscular compression or stretching of the vessel.

Figure 2-B shows similar effects during saline infusion through a femoral vein. Except for the higher level of the basic curves and slight intensification of the phasic cardiac waves, no essential differences occur. Such curves are revealing because they probably reduplicate more nearly conditions in the intact animal.

Figure 2-C shows the effects following hemorrhage. The basal rate of flow was greatly reduced and the right atrium was poorly filled with blood. The cardiac variations in cava flow are still present but of smaller amplitude. Of significance is the fact that despite the collapsed appearance of the vena cava and the low rate of flow, inspiration still acts to increase venous return by approximately the same volume as in figures 2-A and B.

#### CONCLUSIONS

1. Systole and diastole of the heart by creating pressure changes in the atrium cause rhythmic fluctuations in inferior cava flow which are proportional

to these variations. 2. Descent of the diaphragm causes dominantly a marked increase in basal flow in the inferior vena cava which persists even when blood volume is seriously reduced by hemorrhage.

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# COMPARISON OF CHANGES IN INFERIOR CAVA FLOW AFTER HEMORRHAGE AND CIRCULATORY FAILURE FOLLOWING TRANSFUSION

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While reduction in blood volume is undoubtedly the factor most commonly responsible for decreases in venous return, cardiac output and arterial pressure in hemorrhage and shock, it fails to explain why circulatory failure progresses after the states of hypovolemia have become stabilized. Nor does it account for clinical or experimental shock in which significant reduction in blood volume can apparently not be established. Finally, it does not explain the circulatory failure which redevelops in irreversible shock after a state of normovolemia has been restored by substantial infusions of blood or blood substitutes.

Our standard procedure for producing a sustained hypotension by massive hemorrhage, followed by reinfusion of all the blood withdrawn and redevelopment of circulatory failure, provides a method for comparing, in the same animal, the circulatory failure that develops in states of hypo- and normovolemia. The alterations in cardiac action and in the peripheral circulation in these two types of circulatory failure have been reported in various previous communications.<sup>1</sup> This report concerns itself with a comparison of changes of flow in the inferior vena cava during hemorrhagic hypotension and shock and with an analysis of the mechanisms concerned.

**PROCEDURES.** Inferior vena cava flow was determined from optical tracings recorded by the differential flow meter described in a preceding communication (1). Aortic pressures were recorded simultaneously by an optical manometer, and in the later experiments pressures in the right atrium and lower part of the abdominal vena cava were read repeatedly by saline manometers connected to catheters. In order to discount possible effects of respiration and better to control the positions of the Pitot cannula and heart, changes in cava flow were studied in open chest experiments. Animals were maintained by mild but adequate artificial respiration which was interrupted for brief periods when records were taken. If spontaneous breathing supervened measurements were made during apneic pauses during a phase of the cycle when a constant plateau occurred. The reasons for this choice were given in the preceding paper (1). Venous pressure readings were always made at corresponding times of any natural respiration when present.

**RESULTS.** Thirteen experiments were performed on dogs anesthetized with morphine and barbital. The dogs were heparinized after completion of the

<sup>1</sup> A list of publications will be supplied on request by C. J. W.



thoracic operation and so maintained during the entire course of the experiment.<sup>2</sup> The standard bleeding procedure previously described was used (2). Briefly, this consisted in bleeding dogs until mean carotid pressure was reduced to about 50 mm. Hg. This pressure was maintained for 90 minutes, after which it was reduced to 30 mm. Hg by additional small bleedings unless it fell to this level spontaneously. After 45 minutes all the withdrawn blood was reinfused and development of spontaneous circulatory failure awaited. These reactions were of two types; (a) a precipitous progressive decline of arterial pressure to 50 mm. Hg within  $\frac{1}{2}$  to  $1\frac{1}{2}$  hours; (b) an initial period of sustained arterial pressure for an hour or so, followed by a fairly rapid decline. The former seemed to occur in more vulnerable dogs who withstood smaller initial losses of blood; the latter occurred in more resistant animals who yielded larger bleeding volumes before arterial pressure was reduced to 50 mm. Hg or more.

Before analyzing the trends of dynamic events in these two types of animals a few pertinent observations may be mentioned. Nine animals were in good condition after completion of the cannulation. The inferior cava flows ranged from 820 to 1000 cc. per min., with an average of 967 cc. per min. These values compare favorably with average flows, 836 cc. and 930 cc. per min., reported in two series of unanesthetized dogs by Levy and Blalock (3). It may be assumed, therefore, that anesthesia, operation, and technical procedures had not altered the natural flow during the preliminary half hour used to record control data.

The initial bleeding volumes required to reduce pressures to 50 mm. Hg ranged from 14 to 37 cc./kilo. At this pressure level inferior vena cava flows were reduced approximately to half of the control flows, the actual flows ranging from 350 to 575 cc./min., with an average of 443 cc./min.

These and subsequent changes in inferior cava flow in more vulnerable types of dogs are illustrated in figures 1 and 2. In the experiment plotted in figure 1 the initial hypotension ranged nearer 55 mm. Hg and the pressure during the drastic phase, near 40 mm. Hg. Shortly after withdrawal of 270 cc. of blood the inferior cava flow recovered slightly from its initial drop and essentially stabilized at this level except for a temporary rise attributable to a sustaining infusion of saline solution. The most significant changes in arterial pressure consisted in a progressive drop in diastolic pressure (DP) and temporary increase in pulse pressure during augmented inferior cava flow. The decline in diastolic pressure can be explained by the cardiac deceleration which supervened. During the latter part of the 55 mm. period mean arterial pressures had declined to 40 mm. Hg. No further bleeding seemed advisable. During this period inferior cava flow decreased slowly but steadily to a low level of 300 cc. min. just before reinfusion. Reinfusion of the comparatively small volume of withdrawn blood (270 cc.) restored inferior cava flow to the control rate. Systolic arterial pressure exceeded control values, but diastolic pressure remained low owing to the cardiac slowing which supervened. Pressure pulse patterns were essentially restored to normal, but they displayed a large primary spike. However, within

<sup>2</sup> We are indebted to Lederle Laboratories, Inc., Pearl River, N. Y., for the heparin used in these experiments.

10 minutes after infusion arterial pressures and inferior cava flows showed considerable and almost parallel declines, ending in complete circulatory failure within an hour. In experiments of this type, it is obviously impossible to determine whether decrease in venous return caused a reduction of cardiac output which resulted in a decline of arterial pressures or, vice versa, whether pri-

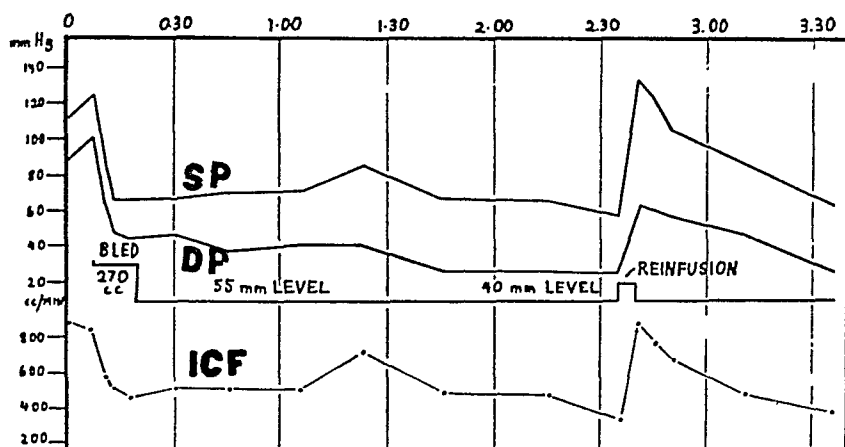


Fig. 1

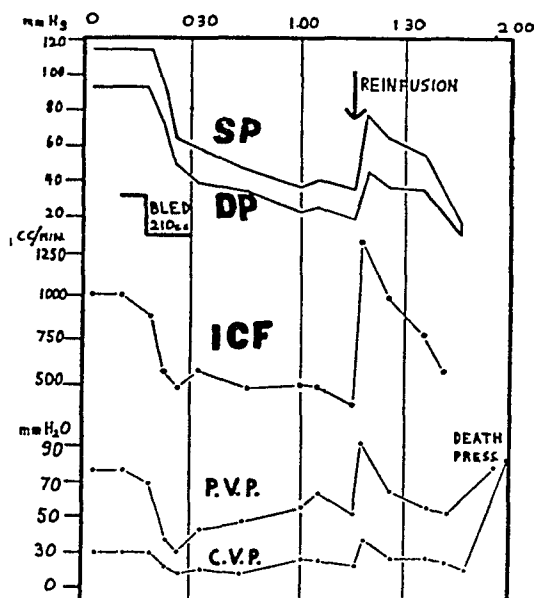


Fig. 2

Figs. 1 and 2. Plots of systolic and diastolic aortic pressures (S.P.-D.P.), inferior cava flow (I.C.F.), peripheral and central venous pressures (P.V.P.; C.V.P.) during hemorrhagic hypotension and precipitous circulatory failure following reinfusion. Discussion in text.

mary reduction in cardiac output and the consequent decrease in arterial driving pressure was responsible for the progressively diminishing cava flow.

An example of a still more precipitous type of circulatory failure in which reinfusion failed to restore arterial pressure which approximated normal is shown in figure 2. The persistent downward trend of arterial pressure after initial bleeding and inability to prevent this by small saline infusions made it necessary to rein-

fuse blood after only 50 minutes of hypotension. In this experiment the downward trend of arterial pressures was accompanied by a slightly diminishing inferior cava flow, but the latter developed with gradually increasing venous pressure gradients and with some recovery in central venous pressure. Moreover, the rate of inferior cava flow was never reduced to levels which were considered critical. Just previous to infusions this was still 380 cc./min. Reinfusion of blood temporarily increased inferior cava flow and venous pressures above normal values, but the blood pressure response was not good. It seems probable that myocardial depression contributed markedly to the course of events. The rapid diminution of inferior cava flow (despite a good venous pressure gradient) combined with a failing myocardium were apparently responsible for the precipitous decline of arterial pressure after infusion. As in the majority of similar experiments reported by Wiggers and Werle (4), central venous pressures decreased during circulatory failure in all experiments of this type in our series. This would seem to indicate that while myocardial depression probably existed, failure of venous return was the dominant factor in reducing cardiac output. There can be little question that in such experiments the more drastic operative procedures contribute to the rapid sequence of circulatory failure after bleeding and reinfusion.

The consecutive dynamic changes in more resistant animals—which more nearly resemble effects obtained in unoperated dogs—are illustrated in figure 3 by actual specimens of records obtained. Curve A is a control record; B and C show the effects of withdrawing 200 and 210 cc. of blood respectively (total 410 cc.). Numerals over flow records indicate reductions of inferior cava flows by  $\frac{1}{3}$  and  $\frac{2}{3}$  respectively. Peripheral and central venous pressures in millimeters saline, which are marked by dotted and solid lines respectively on these records, and tabulated in the legend, indicate that these reductions of inferior cava flow scarcely affect central venous pressure, but markedly reduce peripheral venous pressure, thereby decreasing the pressure gradient in the inferior vena cava. Arterial pressure pulses undergo the deterioration in form previously described. Record D, taken one hour later toward the end of the 50 mm. period reveals a further decline in inferior cava flow, a small increase in the venous pressure gradient, and a slight increase in arterial pressures and pulse pressure. Amazingly, this improvement occurs despite the fact that inferior cava flow has now decreased to rather low levels. We shall return to these apparent inconsistencies. Curve E was taken after withdrawal of an additional 80 cc., making a total bleeding volume of 490 cc. It shows a marked decline of systolic and diastolic pressures. The cardiac slowing, the characteristics of arterial pressure pulse, and the small venous pressure gradient indicate that a return rate of 190 cc. in the inferior cava is about the minimum consistent with maintenance of cardiac action. Curve F shows that a sustaining infusion of saline solution resulted in partial restoration of arterial pressures to safe levels, but inferior cava flow was not increased as a result. The arterial pressures and venous flows shown in this record maintained themselves for another 20 minutes after which the reinfusion of withdrawn blood began.

Record G shows an extremely beneficial effect upon completion of the infusion of 490 cc. of blood. The venous pressures and inferior cava flow were restored to values shown in record A. However, both central and peripheral venous pressures exceed those at the start of the experiment. Successive records taken 8, 10, and 24 minutes later are shown in segments H, I, and J. Obviously, arterial pressures are well sustained, and without other evidence a favorable response appears to exist. However, inferior vena cava flow decreases progressively from 920 to 470 cc., i.e., by about 50 per cent, and the venous pressure gradient in the inferior cava falls progressively from 51 mm. to 5 mm. H<sub>2</sub>O. Likewise, visual inspection of the inferior vena cava during these stages clearly revealed that its diameter steadily decreased. The fact that reduction in inferior cava flow precedes decline of arterial pressures would seem to be conclusive evidence that the diminished driving force of arterial pressures cannot be the fundamental factor responsible for the reduction of inferior cava flow which follows transfusion. The conclusion is generally drawn that some compensatory peripheral mechanism is concerned. We shall return to this inference after completion of our objective analysis. The arterial pressure pulses of records K-L-M indicate the typical progressive circulatory failure generally found in other series of experiments. The inferior cava flow progressively decreases to critical levels shown in the last record. This is accompanied by slight additional reduction in the venous pressure gradient. However, attention may be directed to the fact that central venous pressure in record M is identical with that shown in record A. It is clear that central venous pressures are not indicative of rate of venous flow in the inferior vena cava.

**DISCUSSION.** Two questions require consideration: 1. Is the maintenance of arterial pressures after transfusion while inferior cava flow is decreasing due essentially or dominantly to compensatory increase in total peripheral resistance? 2. Is inferior cava flow an adequate index of total venous return by both cavae, the azygos and the coronary veins?

Returning to curves G to J, they show approximately a 50 per cent reduction in inferior cava flow. It is highly improbable and perhaps impossible that the excellent contour of arterial pressure pulses shown in curve J could be produced with 50 per cent reduction in systolic discharge. Such reduction should yield pressure pulses resembling those in curve B, obtained while inferior cava flow is actually somewhat greater. Systolic pressure is reduced only 4 mm. Hg in curves H to J and the duration of the systolic ejection phase but slightly decreased. Neither is consistent with great reduction of systolic discharge. While systolic pressure declines slightly, diastolic pressure rises progressively in these records. This is partly attributable to an acceleration of the heart from 107/min. in record H to 119/min. in record J. However, careful measurement of the rate of diastolic pressure decline at equivalent pressures in the original curves of H-I-J revealed a progressive retardation indicating increased resistance to run off from the arterial system. However, assuming that marked compensatory increase in total peripheral resistance occurred, this cannot explain the excellent form of the pressure curve. A strong suspicion exists that sys-

tolic discharge and cardiac output were reduced much less than changes in inferior cava flow indicate.

In beginning these studies we felt justified in the belief that changes in inferior cava flow would reveal the proportionate reduction in total venous return. Burton-Opitz (5) had estimated from Stromuhr experiments on cats that the inferior vena cava returns less than 75 per cent of the combined flow in the inferior and superior cavae, but actual recalculations of his data reveal an average of about 53 per cent. Since this does not include blood returned by azygos and coronary systems, it may be inferred that the inferior cava returns less than half of the total venous blood. Levy and Blalock (3) estimated that the inferior cava flow equals about 60 per cent of the cardiac output, i.e., of the total venous return. Furthermore, they found (6) that the dominant decrease in venous return after hemorrhage and intestinal trauma was through the inferior vena cava.

In view of the facts 1, that systolic discharge in segments C-H-I-J does not appear to be reduced as much as the inferior cava flows indicate, and 2, that central venous pressure is identical with that in segment A and even higher in segments H-I-J, the question arises whether the declining inferior cava flow could perhaps be compensated by increased flow from other sources. Of the several possibilities, augmented coronary flow is apparently of some importance. Opdyke (7) who recently investigated coronary flow through the ramus descendens anterior in similar experiments found that the rate of flow increases enormously after arterial pressure is restored by reinfusion (121-420 per cent). This occurred at the time record G of figure 3 was taken. During the succeeding plateau of mean arterial pressure comparable to times when records H-I-J were taken, the flow was still increased about 50 per cent above control values. Assuming this to be typical of flow in all coronary vessels and using data of total flow kindly supplied us by Doctor Gregg, we calculated that an additional 35-50 cc./min. could be returned to the right atrium. This would only increase flow rates to 750 and 520 in segments I and J, respectively. While this does not counterbalance the reduction of inferior cava flow such modest augmentation must help somewhat to sustain filling of the right ventricle, systolic discharge, and maintenance of arterial pressure.

While venous pressure gradients during this experiment were consistent with inferior cava flow changes, the changes in central venous pressure were not. Even during and after the initial bleeding, central venous pressure remained quite constant (A-D); a decline occurred only after additional bleeding leading to drastic reduction in inferior cava flow (E-F). Central venous pressure rose to very high levels after infusion (G), but at the end (M) it was identical with that at the start (A). In their interpretation the possibility of technical accidental errors must be excluded. The method of measurement by use of water manometers is admittedly crude, but recordings were made with care during cessation of artificial respiration, the position of the atrial sound was repeatedly checked and coagulation was not a factor in our heparinized animals. Furthermore, the cannula was repeatedly cleared by small infusions of saline and the meniscus allowed to seek its own level. Moreover, since the animal was placed

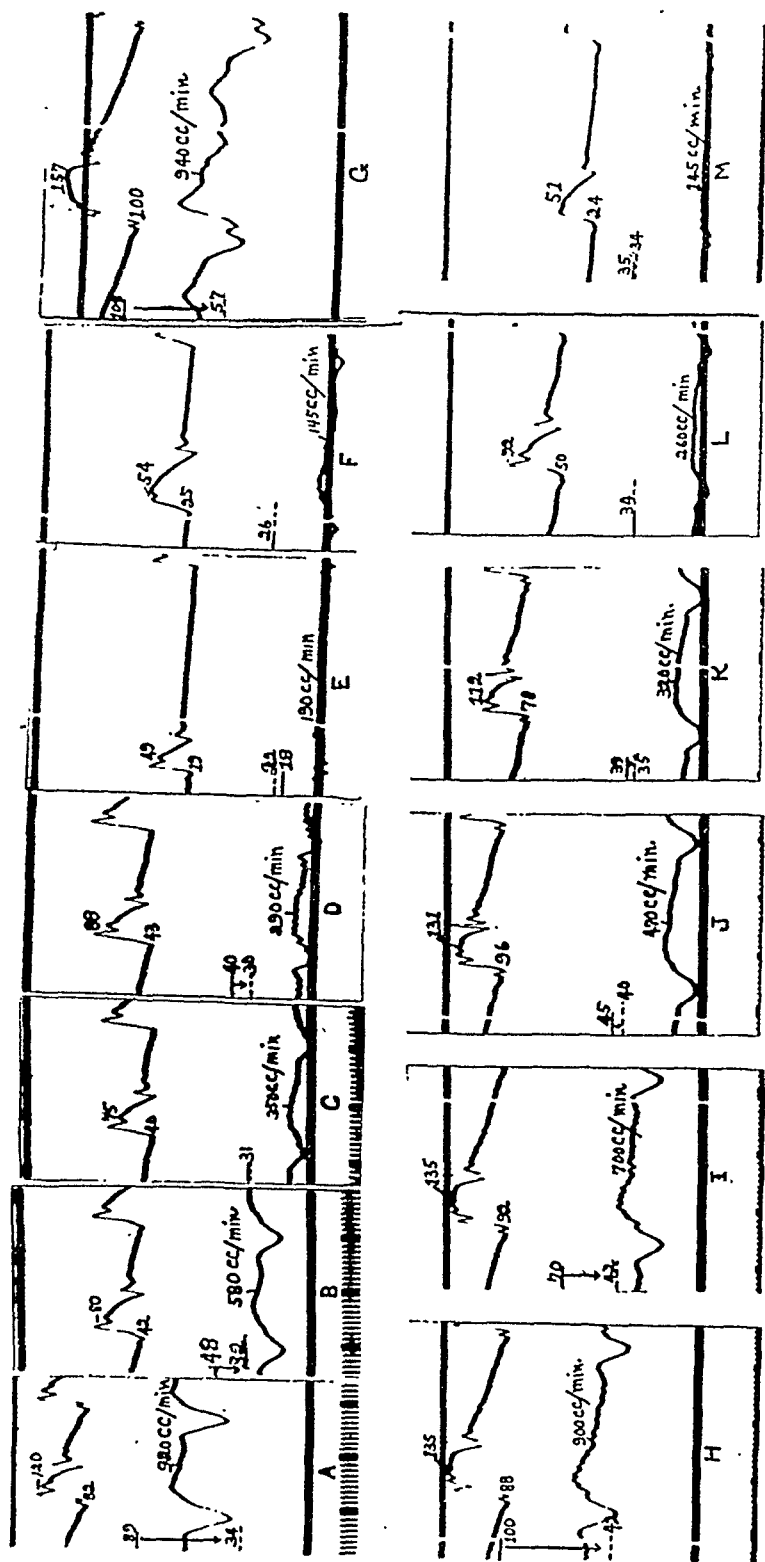


Fig. 3. Segments of records showing aortic pressures (upper) and flow rates in inferior cava (lower) during hemorrhagic hypotension, A-F; after reinfusion, G; and during subsequent circulatory failure, H-M.

TIME	VENOUS PRESS. GRADIENT	BASAL FLOW	TIME	VENOUS PRESS. GRADIENT	BASAL FLOW
A-11.39	89 > 34	920	H-2.20	100 > 42	900
B-11.44	48 > 32	580	I-2.22	70 > 42	700
C-12.11	31 > 31	350	J-2.36	45 > 40	470
D-1.14	40 > 30	290	K-2.42	39 > 35	320
E-1.29	22 > 18	190	L-2.48	34 > 34	280
F-1.36	26 > 26	145			
G-2.12	108 > 57	940	M-3.02	35 > 34	145

on its left side and with purposeful translocation of the heart to the left, we avoided impacts of a relatively empty heart which might result in traction or compression of the right auricle sufficient to elevate pressures beyond dynamic values established.

The dynamic factors that might increase central venous pressure over that anticipated from inferior cava flow are (a) myocardial depression with inadequate removal of blood from the right atrium, or (b) compensatory increase in flow from other regions. In previous communications the first factor has been stressed on the basis of various cardiodynamic studies. It apparently enters in different degrees at different periods of an experiment and varies considerably in different dogs. It cannot be excluded in the rapid post-infusion failure of experiments exemplified by figures 1 and 2, or in the final stages of the experiment of figure 3, L-M. It is less likely to be a factor in maintaining central venous pressures in records H-I-J than the compensatory flow factor. However, both may be concerned.

#### SUMMARY

The inferior cava flow recorded by a differential pressure flow meter decreases during sudden hemorrhage and remains low during the 50 and 30 mm. Hg periods of hypotension maintained in our standard experiments. A blood flow of about 150 cc./min. is approximately the lowest critical level which sustains the heart and circulation in a 10 to 12 kilo dog. The decrease in flow rate is accompanied by a significant reduction in venous pressure gradient between the lower end of the inferior cava and the right atrium. It also causes a decline of atrial pressure. However, when the flow is reduced for an hour or more and when it approaches the critical rate, central venous pressures tend to increase somewhat, while flow decreases.

These effects of simple hemorrhage on inferior cava flow were contrasted in the same animal with changes which supervene after reinfusion of all withdrawn blood and subsequent spontaneous circulatory failure. Reinfusion immediately restored inferior cava flow to normal values as a result of a high peripheral venous pressure. This increased flow plus a tremendous augmentation of coronary flow raises right atrial pressure, augments cardiac output, and improves the form of central arterial pressure pulses, as well as restores arterial pressures. However, flow rate in the inferior cava progressively diminishes, as does the differential venous pressure; indeed, a 50 per cent reduction may occur before central arterial pressure pulses show significant changes in form or quantitative values. This clearly indicates that reduction of inferior cava flow is an antecedent factor in circulatory failure after transfusion. Contrary to effects following simple reduction of blood volume by hemorrhage, the reduction in inferior cava flow does not eventuate in as great a reduction of right atrial pressure; indeed, right atrial pressure was often as high at the end as at the start. In short, in hemorrhagic shock reduction in inferior cava flow correlated with decrease in pressure gradient, but not with changes in right atrial pressure.

This may be due to compensated flow from other venous sources including the coronary veins, but is more probably due to myocardial depression which is a subsidiary factor causing the circulatory failure.

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